PYY(3–36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity

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Vrang, Niels, Andreas Nygaard Madsen, Mads Tang-Christensen, Gitte Hansen, and Philip Just Larsen. PYY(3–36) reduces food intake and body weight and improves insulin sensitivity in rodent models of diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 291: R367–R375, 2006. First published April 13, 2006; doi:10.1152/ajpregu.00726.2005.—The gut hormone peptide YY (PYY) was recently proposed to comprise an endogenous satiety factor. We have studied acute anorectic functions of PYY(3–36) in mice and rats, as well as metabolic effects of chronic PYY(3–36) administration to diet-induced obese (DIO) mice and rats. A single intraperitoneal injection of PYY(3–36) inhibited food intake in mice, but not in rats. We next investigated the effects of increasing doses (100, 300, and 1,000 µg·kg⁻¹·day⁻¹) of PYY(3–36) administered subcutaneously via osmotic minipumps on food intake and body weight in DIO C57BL/6J mice. Whereas only the highest dose (1,000 µg·kg⁻¹·day⁻¹) of PYY(3–36) significantly reduced food intake over the first 3 days, body weight gain was dose dependently reduced, and on day 28 the group treated with 1,000 µg·kg⁻¹·day⁻¹ PYY(3–36) weighed ~10% less than the vehicle-treated group. Mesenteric, epididymal, retroperitoneal, and inguinal fat pad weight was dose dependently reduced. Subcutaneous administration of PYY(3–36) (250 and 1,000 µg·kg⁻¹·day⁻¹) for 28 days reduced body weight and improved glycemic control in glucose-intolerant DIO rats. Neither 250 nor 1,000 µg/kg PYY(3–36) elicited a conditioned taste aversion in male rats.

diet-induced obese rat; peptide YY; taste aversion; indirect calorimetry; locomotor activity

PEPTIDE YY (PYY) is a 36-amino acid peptide that belongs to the PP-fold peptide family, together with pancreatic polypeptide and neuropeptide Y (15). PYY is found in the circulation as PYY(1–36) and PYY(3–36) (16, 17), the latter of which is produced from PYY(1–36) by dipeptidyl peptidase IV activity (26). Circulating PYY originates from intestinal L cells lining the gut, and PYY is released postprandially, particularly after ingestion of fat (3). PYY(1–36) predominates in the fasted state, whereas PYY(3–36) predominates after a meal (16, 17).

Peripheral administration of PYY predominantly inhibits digestive processes. Systemic administration of PYY has been shown to inhibit gastric emptying and acid secretion, reduce stimulated pancreatic exocrine secretion, and increase intestinal transit time (4, 5, 28, 29). Interest in the function of L cell-derived PYY(3–36) was renewed recently by a report demonstrating potent anorectic effects of exogenously administered PYY(3–36). Intravenous infusion of PYY(3–36) for 90 min to healthy (7) and obese (6) humans reduced appetite and caloric intake for the subsequent 24 h, indicating a possible therapeutic role of PYY(3–36) in appetite and weight control.

The anorectic properties of peripherally administered PYY(3–36) were, however, questioned in a publication summarizing a wealth of in vivo data from a large number of independent laboratories (32). In this study, PYY(3–36) was administered at 3–1,000 µg/kg to several rodent species and shown to have, at best, modest and transient effects on food intake and body weight, in notable contrast to the initial study by Batterham and coworkers (7). Recently, however, several studies have shown acute reductions in food intake after intraperitoneal (2, 19) or intravenous (12, 31) administration of PYY(3–36). Also, chronic administration of PYY(3–36) has been shown to reduce body weight in ob/ob mice, obese Zucker rats, and diet-induced obese (DIO) mice (30), and intermittent (1 h on and 1 h off) intravenous infusion of PYY(3–36) for 10 days was reported to produce long-lasting reductions in food intake and body weight (11), further supporting the anorexic properties of this gut hormone.

The present experiments were designed to unravel the putative anorectic and/or body weight-lowering effects of PYY(3–36) and the discrepancies found in the literature with use of acute and chronic administration and different rodent models of DIO. In addition, we wanted to investigate PYY(3–36)-mediated effects on glucose homeostasis in DIO mice and rats displaying impaired glucose tolerance.

METHODS

Animals. All experiments were conducted in accordance with internationally accepted principles for the care and use of laboratory animals and were approved by the Danish Committee for Animal Research. Studies were carried out in male NMRI and C57BL/6J mice, male Wistar and Sprague-Dawley rats (Charles River Laboratories), and male DIO Sprague-Dawley rats (Rheoscience breeding colony). Studies were performed in the In Vivo Pharmacology Department of Rheoscience. Animals were kept on a 12:12-h light-dark cycle (lights on at 0600) in a temperature-controlled environment (22–24°C) with free access to food and water unless otherwise stated.

Peptides. Human PYY(3–36) was purchased from Bachem (catalog no. H-8585, batch 0556494) and Phoenix Pharmaceuticals (batch 419701), and a third independent batch was acquired from Zealand Pharmaceuticals (Glostrup, Denmark). All peptides were certified and analyzed by HPLC; purity of all peptides was >97%. Vehicle for all experiments was isotonic saline. PYY(3–36) synthesized at Zealand Pharmaceuticals (code ZP1740T) was used for the chronic dosing experiments and the taste aversion experiment. Stability of PYY(3–36) in solution was initially confirmed in vitro at 37°C. Briefly, a 0.25 mg/ml solution of PYY(3–36) was prepared in saline and stored at 37°C. From this solution, samples were taken at days 1, 3, 7, 8, 10, 11, 14, 16, 18, 21, 23, and 25. Degradation was stopped by precipitation of sample with 10 µl of 50:50 (vol/vol) MeCN-trifluoroacetic acid.

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Nutella diet consists of a 1:1 (wt/wt) blend of Nutella and Altromin 1324. HF, high fat; CHO, carbohydrate.

Samples were analyzed using HPLC and UV detection at 215 nm. The calculated degradation rate was <0.5%/day.

**General procedure.** On arrival in the laboratory, the animals were housed in a group for 1 wk to allow for acclimatization (3 rats per cage, 5 mice per cage) and then transferred to individual cages for the remainder of the experiments. All animals were singly housed for ≥7 days before PYY(3–36) injection. The animals were accustomed to the injection procedure by intraperitoneal injection with saline (0.5 ml for rats and 0.2 ml for mice) at 0900 for 3 days before PYY(3–36) administration. The animals were randomized into groups according to body weight on the day before the first PYY(3–36) injection. Food was removed at 1500, and the animals were fasted for the subsequent 19 h (water was available ad libitum). After the animals were injected with the test substances at 0900 on the following day, a preweighed amount of food was returned to the animals. The food was weighed 1, 2, and 3 h and, for some experiments, 24 h after the injection. For 3 days after the first injection, the animals were injected daily at 0900 with vehicle and the procedure was repeated (overnight fast and injection with compound on the following day). No animals were injected with PYY(3–36) more than twice, and randomization was repeated such that no animal received the same test substance more than once.

**Experiment 1: PYY(3–36) in Wistar rats.** Twenty-four male Wistar rats (Charles River) weighing ~100 g on arrival were randomized into three treatment groups (n = 8 each): vehicle, 100 μg/kg PYY(3–36) from Bachem, and 100 μg/kg PYY(3–36) from Phoenix. The animals were fed pelleted chow (Altromin 1324, Petersen, Ringsted, Denmark; Table 1).

**Experiment 2: PYY(3–36) in NMRI mice.** A total of 48 male NMRI mice (Charles River) weighing 25–30 g on arrival were used in two different experiments. The first set of mice (n = 24) was randomized into three treatment groups (n = 8 each): vehicle, 100 μg/kg PYY(3–36) from Bachem, and 100 μg/kg PYY(3–36) from Phoenix. The experiment was repeated once. Because of food spillage between hours 3 and 24, a second experiment was performed in 24 mice, which were given sturdier pellets (D12489B, Research Diets; Table 1). These mice were randomized into four treatment groups (n = 6 each): vehicle, 10 μg/kg PYY(3–36) from Bachem, 100 μg/kg PYY(3–36) from Bachem, and 1,000 μg/kg PYY(3–36) from Bachem.

**Experiment 3: PYY(3–36) in lean and DIO C57BL/6J mice.** Because it has been reported that PYY(3–36) is more effective in reducing food intake in DIO mice (8), the effect of PYY(3–36) on lean chow-fed and obese chocolate (Nutella)-overfed C57BL/6J mice was studied. Thirty male C57BL/6J mice (Charles River), 4–5 wk old at the time of arrival at Ledøje, were kept five per cage for 5 wk and weighed weekly. All mice had ad libitum access to standard chow (Altromin 1324). In addition to chow, half of the mice were offered a 1:1 mixture of chow and a calorie-dense chocolate spread (Nutella; Table 1). Fresh Nutella was provided biweekly. Mice were weighed once weekly. On day –7, the mice were transferred to individual cages (still with free access to food and water), where they were kept for 7 days. The mice were randomized into four treatment groups: chow-fed vehicle (n = 7), chow-fed 100 μg/kg PYY(3–36) from Zealand Pharmaceuticals (n = 8), Nutella-fed vehicle (n = 7), and Nutella-fed 100 μg/kg PYY(3–36) from Zealand Pharmaceuticals (n = 8).

**Experiment 4: chronic subcutaneous administration of PYY(3–36) to DIO male C57BL/6J mice.** Forty male 8-wk-old C57BL/6J mice (Charles River) were housed five per cage for 4 wk and then transferred to individual cages for 1 wk. Throughout this period, the mice were offered standard chow (Altromin 1324) as well as a 1:1 mixture of chow and Nutella. Body weight was recorded on days −10, −3, and 0. Body weights on day 0 were used to randomize mice into four groups (n = 10 each): vehicle, 100 μg·kg⁻¹·day⁻¹ PYY(3–36), 300 μg·kg⁻¹·day⁻¹ PYY(3–36), and 1,000 μg·kg⁻¹·day⁻¹ PYY(3–36). Compounds were administered via osmotic minipumps (model 2004, Alzet; 200 μl, 0.25 μl/h, 28 days of delivery). The pumps were filled 24 h before implantation and primed in 0.9% NaCl at 37°C. On day 1, the mice were anesthetized by isoflurane inhalation, and the pumps were implanted subcutaneously in the lower back. After the operation, the mice were allowed to recover and then returned to their cages, which contained preweighed chow and Nutella mix (fresh Nutella mix was provided biweekly). Chow and Nutella intake, as well as body weight, were recorded biweekly for the remainder of the study. On day 25, the animals were randomized to participate in an oral glucose tolerance test (OGTT) on day 26 or 27 (5 mice per group per day). At 18 h before the OGTT, the animals were transferred to new cages, in which they had access to 50% of their average daily food consumption. These semistarved mice were subjected to an OGTT at 0800 on days 26 and 27. Blood samples were taken from the tail, and blood glucose was measured using strips (Ascensia Contour, Bayer Health Care) at 10 min before, time 0, and 15, 30, 60, and 120 min after oral administration of 2 g/kg glucose (using 1 g/ml distilled H₂O, given at time 0). The oral glucose load was given as gavage via a gastric tube connected to a syringe to ensure accurate dosing. After termination of the OGTT, the mice were killed by CO₂ and decapitation, and fat depots (retroperitoneal, inguinal, epididymal, and mesenteric) were dissected and weighed.

**Experiment 5: chronic subcutaneous administration of PYY(3–36) to DIO male rats.** Selectively bred 3- to 5-wk-old male rats displaying enhanced likelihood of developing DIO [bred on a Sprague-Dawley background (24)] were transferred from Rhescoience breeding facilities to the Department of In Vivo Pharmacology. A total of 48 DIO rats were included in the study. After selection for the study, the animals were housed individually and offered an energy-density high-fat diet (catalog no. 12266B, Research Diets; Table 1) and water ad libitum.

After 12 wk on the high-fat diet, the animals were stratified according to body weight (day −1) to participate in one of the following drug treatment studies (n = 12 each): vehicle, 25 μg·kg⁻¹·day⁻¹ PYY(3–36), 250 μg·kg⁻¹·day⁻¹ PYY(3–36), and 1,000 μg·kg⁻¹·day⁻¹ PYY(3–36). The compounds were administered via osmotic minipumps (model 2ML4, Alzet; 2 ml, 2.5 μl/h, 28 days of delivery), which were filled on day −1 and “primed” overnight as described for experiment 4. On day 0, the animals were anesthetized by isoflurane inhalation, and the pumps were implanted subcutaneously in the lower back. After the operation, the DIO rats were allowed to recover and then returned to their cages. Rimadyl (Carprofen, Pfizer) was administered once for analgesia. Food intake and body weight were recorded daily for the first 7 days and then for the remainder of the study once weekly. Indirect calorimetry was performed at thermoneutrality (31°C) in calorimetry cages (TSE System) on days 14–17 (8 animals per day). On the day before the test, eight animals (randomly chosen across groups) were transferred to a temperature-controlled (29°C) room to acclimate to the new surroundings. On the morning of the next day, the rats were weighed, and blood pressure and heart rate were measured from the tail vein by an indirect tail-cuff method with photoelectric sensors (single-sensor NIBP system, model 29-SSP, IITC Life Science). Briefly, the rats were familiarized by a skilled animal technician with gentle manual restraint and tail-cuff inflation to 200 mmHg daily for 4 days before

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<th>Diet</th>
<th>Expt No.</th>
<th>Caloric Density, kcal/g</th>
<th>Fat, %</th>
<th>CHO, %</th>
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Table 1. **Diet**

**Caloric Density**, % energy

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the experiment. On the morning of the experiment, immediately after
the animals were removed from the temperature-controlled room, the
tail-cuff measurement was performed. A maximum of three inflations
were performed to obtain a successful recording from the photoelectric
sensor. Systolic blood pressure and heart rate were determined
using the Power Lab (MacLab 4.0) data acquisition system with Chart
4.5 software (AD Instruments). After the tail-cuff experiment, the rats
were transferred to airtight Plexiglas cages (no food, no bedding, ad
libitum water). Airflow into and out of the cages was controlled by the
calorimetry system. O₂ consumption (V̇O₂) and CO₂ production (VCO₂)
were measured every 20 min over the next 6 h; data from the first 2 h of
the test (i.e., the acclimatization period) were excluded from analysis.
Respiratory exchange ratio (VCO₂/V̇O₂) and heat production (kcal·kg body wt⁻¹·h⁻¹) were calculated for the remaining
4-h period. After experimentation, the rats were returned to their home
cages and given free access to food and water.

On days 27 and 28 (half the animals from each group per day), the rats
were subjected to an OGTT before they were killed by CO₂
anesthesia. At the time of death, a blood sample from the retroorbital
plexus was collected for subsequent PYY(3–36) analysis. The OGTT
was carried out at 0800. The animals were mildly fasted, inasmuch as
they had access to only 50% of their daily energy requirements in the
preceding 20 h (since 1200 on the previous day). Blood samples were
taken from a tail vein, and blood glucose was measured using strips
(Escambia Contour) at 30 min before, time 0, and 15, 30, 60, 90, 120,
and 180 min after oral administration of 2 g/kg glucose (using 2 g/ml
distilled H₂O). The oral glucose load was given as gavage via a gastric
tube connected to a syringe to ensure accurate dosing. Plasma insulin
was measured in duplicate at 0, 15, 30, 60, and 120 min by an
ultrasensitive ELISA method (Diamyd Diagnostics). A 500-μl baseline
blood sample taken from the tail vein 30 min before glucose
administration was used for analysis of insulin, triglycerides, and
cholesterol; triglycerides and cholesterol were measured using an
automated analyzer (model DT600i, Orthochronical Diagnostics, John-
son and Johnson). For insulin analysis, 200 μl of blood were sampled
per time point. Hence, a total of 1.5 ml of blood was sampled for
hormone/lipid analyses in addition to the 5–10 μl of blood per time
timepoint used for the glucose measurements.

The insulin sensitivity index was calculated according to Matsuda
and DeFronzo (25) as

\[
\frac{10,000}{\sqrt{(\text{FPG} \times \text{FPI}) \times (\text{meanOGTT}_{\text{glucose}} \times \text{meanOGTT}_{\text{insulin}})}
\]

where FPG and FPI are fasting plasma glucose and insulin, respec-
tively, and mean OGTT is the area under the curve (AUC) for glucose
and insulin excursions.

Human PYY(3–36) was measured in terminal plasma samples
using a commercially available RIA kit (catalog no. PY-67HK,
Linco Research).

Experiment 6: taste aversion and locomotor activity after a single
intraperitoneal injection of PYY(3–36). From the date of arrival, 40
male Sprague-Dawley rats (~250 g) were kept under a reverse
12:12-h light-dark cycle with lights on at 0130 and off at 1330 and in
temperature- and humidity-controlled rooms. The animals were given
10 days to synchronize to this new light-dark cycle before they were
transferred to single locomotor activity cages (standard Nalgene rat
cages mounted with 4 sets of photo cells) with ad libitum access to
food and one bottle of water. The rats were accustomed to the
injection paradigm by daily handling and mock injections on days −5,
−4, −3, and −2 (0.9% saline, pH 7.4, 3 ml/kg ip). On day 0, the animals
were randomized according to body weight into four treat-
ment groups (n = 10 each): vehicle (0.9% saline), 250 μg/kg PYY(3–36),
and 1,000 μg/kg PYY(3–36) + 80 mg/g LiCl. Food and water
were removed from the cages at 0700, and 2 h later, the animalswere
offered a preweighed bottle containing 0.1% saccharin-flavored water.
Before lights out (1330), the saccharin bottles were removed and
weighed, the rats were dosed with the compounds and fed, and plain
water was returned to the cages. Locomotor activity was recorded as
consecutive beam breaks for 18 h after the injection; data were
collected at 15-min intervals, and significance was tested 6, 12,
and 17 h after drug administration by one-way ANOVA and Fisher’s post
hoc test. At 72 and 96 h after dosing, the rats were exposed to a
two-bottle taste preference assay: a choice between tap water and the
0.1% saccharin solution given before testing. Water and saccharin
intake was measured for 12 h on the two occasions, and the ratio of
saccharin to total liquid intake was calculated.

Statistics. Values are means ± SE unless otherwise stated. Statis-
tical evaluation of the data was carried out using one-way ANOVA
with Fisher’s post hoc analysis. P < 0.05 was considered statistically
significant. For experiments 4 and 5, body weight and food intake
groups were compared on days 0, 4, 14, and 25.

RESULTS

Experiments 1–3: acute feeding. We examined the anorectic
effects of PYY(3–36) on mice and rats fed different diets (Table 1). PYY(3–36) from three different sources was tested in
acclimatized animals that had been fasted for 19 h (from
1500) and injected at 0900 on the following day. The results
from the acute studies are shown schematically in Table 2.
Intraperitoneal administration of PYY(3–36) had no effect on fasting-induced feeding in male Wistar rats. However, using
the same experimental design and the same preparations of
PYY(3–36) that were used in the rat study, we were able to
demonstrate an ~30% reduction in 3-h food intake in male
NMRI mice (Table 2). To further investigate the appetite-
suppressive effects of PYY(3–36) and to overcome problems
with chow spillage at hour 24, we repeated the mouse exper-
iment using the sturdier pellets from Research Diets and
increasing doses (10, 100, and 1,000 μg/kg body wt) of
PYY(3–36). Surprisingly, only the highest dose significantly
reduced food intake, and only at hour 1. Because it has been
reported that the anorectic effect of PYY(3–36) is increased in
DIO C57BL/6 mice (8), we next tested whether PYY(3–36)
reduces caloric intake in lean and obese C57BL/6J mice.
Whereas PYY(3–36) transiently reduced food intake in lean
C57BL/6J mice, no effects were seen in the DIO Nutella-
overfed mice (Table 2). Because of the equivocal results
obtained from the acute experiments and the relatively sparse
information with respect to plasma half-life of PYY(3–36) in
rodent plasma, we decided to investigate the effects of chronic
infusion of PYY(3–36) on food intake and body weight, as
well as possible associated effects, on glucose homeostasis in
two different animal models of DIO.

Experiment 4: chronic administration of PYY(3–36) to DIO
C57BL/6J mice. PYY(3–36) caused a dose-dependent reduc-
tion in body weight (Fig. 1A). Body weight was statistically
analyzed on days 4, 14, and 25. On day 4 mice treated with 300
and 1,000 μg·kg⁻¹·day⁻¹ PYY(3–36) weighed significantly
less than vehicle-treated mice, and on day 14 all three PYY(3–36)-
treated groups were significantly lighter than the vehicle-
treated group. On the day before the OGTT (day 25), body
weight gain was ~10% less in mice receiving the highest doses
of PYY(3–36) than in vehicle-treated mice: 108.5 ± 1.3,
104.6 ± 1.4, and 101.0 ± 1.9% for 100, 300, and 1,000
μg·kg⁻¹·day⁻¹ PYY(3–36), respectively, vs. 112.2 ± 1.9%
for vehicle (P < 0.05 vs. vehicle). Reduced food intake was
observed on day 4 in mice treated with the highest dose of

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PYY(3–36) (Fig. 1B). The apparent increase in food intake on day 14 was due to a supplementation in food at day 11: the novelty of fresh chow increases the exploratory activity (grinding and chewing) of mice (unpublished observations). The time course of the blood glucose levels after the oral glucose load is shown in Fig. 2A. As a measure of glucose tolerance, the AUC (0–180 min) was used (Fig. 2B). On the day the animals were killed, body fat compartments were dissected and weighed (Fig. 3). Compared with vehicle, continuous subcutaneous infusion of 1,000 μg·kg⁻¹·day⁻¹ PYY(3–36) significantly reduced the size of all four fat compartments, as well as the total size of the dissected fat depots (Fig. 3).

**Experiment 5: chronic subcutaneous administration of PYY(3–36) to DIO male rats.** Because of the potent body weight-lowering effects of PYY(3–36) observed in the chronic mouse study, we used 25, 250, and 1,000 μg·kg⁻¹·day⁻¹ PYY(3–36) in the rat study. Figure 4 shows the body weight and daily food intake over the course of the study. A rapid drop in body weight was observed over days 1–4 (Fig. 4A). Food intake was dramatically suppressed on days 1–3 and gradually returned toward baseline levels, which were reached on day 7 (Fig. 4B). To determine possible thermogenic and/or autonomic effects of PYY(3–36), we performed indirect calorimetry and noninvasive blood pressure determinations on days 14–17 (8 rats per day). PYY(3–36) failed to induce significant changes in basal metabolic rate or respiratory exchange ratio (Fig. 5). Rats treated with 250 μg·kg⁻¹·day⁻¹ PYY(3–36) showed a reduction in systolic blood pressure on days 14–17: 140 ± 5, 113 ± 6, and 123 ± 5 mmHg at 25, 250, and 1,000 μg·kg⁻¹·day⁻¹, respectively, vs. 132 ± 6 mmHg with vehicle (P < 0.05, 250 μg vs. vehicle). Heart rate did not differ between groups on days 14–17: 335 ± 13, 336 ± 8, and 345 ± 15 beats/min at 25, 250, and 1,000 μg·kg⁻¹·day⁻¹ PYY(3–36), respectively, vs. 347 ± 13 with vehicle (P < 0.05, 250 μg vs. vehicle).

On days 26 and 27 (before the OGTT), blood from semi-fasted rats was sampled for determination of baseline insulin, triglycerides, and cholesterol. PYY(3–36) levels were determined from a terminal blood sample by an assay specific for human PYY(3–36) to ensure specific detection of exogenous PYY(3–36). Baseline insulin was lower in the DIO rats treated with the highest dose of PYY(3–36) (Table 3), whereas the reductions in cholesterol and triglycerides in PYY(3–36)-treated rats were not significant (Table 3). Plasma levels of human PYY(3–36) immunoreactivity in rats treated with 25 μg·kg⁻¹·day⁻¹ were similar to those of vehicle-treated rats, whereas 250 and 1,000 μg·kg⁻¹·day⁻¹ gave rise to readily detectable levels of human PYY(3–36), demonstrating that osmotic minipumps were effectively delivering the drug at the termination of the experiment (Table 3). The time course of blood glucose levels after the oral glucose load is shown in Fig. 6A, and blood insulin levels are shown in Fig. 6B. The resulting integrated glucose and insulin excursions were used to evaluate glycemic control (Table 4). The AUC for glucose was reduced, but not significantly, in the DIO rats treated with 250 and 1,000 μg·kg⁻¹·day⁻¹ PYY(3–36); however, clear effects were seen in the AUC for insulin (Table 3). Interestingly, also rats treated with the lowest dose of PYY(3–36) showed a decreased AUC for insulin, despite a body weight curve very similar to that of vehicle-treated rats (Fig. 4). As a measure of whole body insulin sensitivity, the insulin sensitivity index was calculated according to Matsuda and DeFronzo (25). Insulin sensitivity was marginally improved in DIO rats treated with the lowest dose (250 μg·kg⁻¹·day⁻¹) of PYY(3–36) and markedly improved in rats treated with the highest dose (1,000 μg·kg⁻¹·day⁻¹; Table 4).

### Table 2. Acute effects on food intake in rats and mice

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<td>100 μg/kg PYY(3–36) (Phoenix)</td>
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<td><strong>Expt 2A: NMRI mice</strong></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>Altromin 1324</td>
</tr>
<tr>
<td>100 μg/kg PYY(3–36) (Bachem)</td>
<td>Altromin 1324</td>
</tr>
<tr>
<td>100 μg/kg PYY(3–36) (Phoenix)</td>
<td>Altromin 1324</td>
</tr>
<tr>
<td><strong>Expt 2B: NMRI mice</strong></td>
<td></td>
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<tr>
<td>Vehicle</td>
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</tr>
<tr>
<td>10 μg/kg PYY(3–36) (Bachem)</td>
<td>HF - D12489B</td>
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<tr>
<td>1,000 μg/kg PYY(3–36) (Bachem)</td>
<td>HF- D12489B</td>
</tr>
<tr>
<td>1,000 μg/kg PYY(3–36) (Zealand)</td>
<td>HF- D12489B</td>
</tr>
<tr>
<td><strong>Expt 3: C57BL/6J mice</strong></td>
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<tr>
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<tr>
<td>100 μg/kg PYY(3–36) (Zealand)</td>
<td>Altromin 1324</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Nutella</td>
</tr>
<tr>
<td>100 μg/kg PYY(3–36) (Zealand)</td>
<td>Nutella</td>
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</table>

Values are means ± SE. Peptide YY [PYY(3–36)] was administered intraperitoneally at 0900 after a 19-h fast. Food intake was recorded 1, 2, 3, and 24 h after injection of PYY (3–36) or vehicle. ND, not determined. *P < 0.05 vs. vehicle (ANOVA followed by Fisher’s post hoc analysis).
Experiment 6: taste aversion and locomotor activity after a single intraperitoneal injection of PYY(3–36). To investigate whether part of the anorectic effect of PYY(3–36) is due to malaise or induction of nonspecific general behavioral suppression, the ability of a single injection of PYY(3–36) to elicit a conditioned taste aversion response was examined in male Sprague-Dawley rats. LiCl was used as a positive control. A single intraperitoneal injection of PYY(3–36) did not elicit taste aversion, whereas the rats injected with 80 mg/kg LiCl showed a strong reduction in saccharin preference ratio: saccharin preference ratio at the second test session (hours 96–108) was 32.5/H11006110.7% for 80 mg/kg LiCl, 89.2/H110063.4% for 250 g/kg PYY(3–36), and 81.7/H110065.1% for 1,000 g/kg PYY(3–36) vs. 91.7/H110062.1% for vehicle (Fig. 7). Locomotor activity was recorded as consecutive beam breaks in the same experiment; whereas LiCl slightly reduced general locomotor activity, locomotor activity in the dark (activity) phase increased slightly after PYY(3–36) injection (Fig. 7B).

DISCUSSION

Our results demonstrate that continuous subcutaneous administration of PYY(3–36) dose dependently reduces body weight in two rodent models of DIO. In the DIO rat model displaying several hallmarks of the human metabolic syndrome (hyperinsulinemia, hyperglycemia, and dyslipidemia), 28 days of PYY(3–36) treatment produces an ~10% decline in body weight gain, as well as an amelioration of glucose tolerance and insulin sensitivity, indicating the usefulness of PYY(3–36) or other Y2 receptor ligands in the pharmacological treatment of obesity.

Although the results from the 28-day chronic dosing studies are in agreement with previously reported findings that twice-daily injections of PYY(3–36) reduce cumulative food intake and body weight gain (7), the effects reported in the present study are of greater magnitude, underscoring the importance of continuous delivery of PYY(3–36) to obtain maximal effect. The magnitude of the body weight-lowering effects observed...
in the present study is more comparable to the weight loss recently reported as a result of continuous osmotic minipump delivery of PYY(3–36) to ob/ob mice, obese Zucker rats, and DIO mice (30). In addition, the effect of 4 wk of continuous PYY(3–36) administration to prediabetic Zucker diabetic fatty (ZDF) rats was investigated, and dose-dependent reductions in food intake and improvements in glycemic indexes (reduction in HbA1c and fructosamine) that were independent of effects on body weight were found (30). However, the rapid and uneven development of frank diabetes in the ZDF rat, leading to urinary loss of glucose and, hence, energy, complicates interpretation of these results. Whereas Zucker rats are insensitive to leptin signaling, this important hormonal signal of total energy resources is maintained in DIO rats (22, 23). As such, the DIO rat used in the present study represents a far better model of human obesity and metabolic syndrome than the monogenetic obesity/diabetes models. The DIO rat strain used in the present study has been extensively characterized and shares a number of traits with human obesity, including polygenic inheritance (24), insulin resistance (24), hyperleptinemia, and central leptin resistance (22, 23). The potent appetite-suppressive and body weight-lowering effects and accompanying amelioration of insulin resistance in the present study with use of the well-characterized DIO rat as a model for the human obesity syndrome, therefore, fits well with the recent observations from human studies that PYY(3–36) effectively suppresses appetite in lean and obese individuals (6, 7). The data from the chronic study suggest that chronic stimulation of Y2 receptors is a powerful tool to reduce body weight. The body weight loss after PYY(3–36) administration to DIO rats observed in the present study is similar in magnitude to that observed in rats after administration of the mixed serotonin and norepinephrine reuptake inhibitor sibutramine (10) and the cannabinoid receptor antagonist rimonabant (14).

Curiously, the meager and unpredictable effects of acutely administered PYY(3–36) on feeding are in stark contrast to the robust effects on energy homeostasis seen on chronic PYY(3–36) administration to mice and rats. Despite application of a research protocol identical to that reported by Batterham and coworkers (7), we were unable to detect acute anorectic effects in male rats. Different batches of PYY(3–36) administered to different strains of mice fed different diets gave rise to short-duration reductions in food intake. The lack of clear-cut acute effects on food intake is similar to findings recently reported by several research laboratories that were unable to replicate the initial findings of anorectic and body weight-lowering effects of acute or chronic administration of PYY(3–36) to rodents (32). Although the exact reason(s) for the discrepancies is difficult to reconcile, the necessity of long habituation periods was recently demonstrated by a report showing that PYY(3–36)-induced anorexia was absent in naive mice but present in 7-day-acclimatized mice (19). In our acute experiments, mice and rats were acclimatized for ≥3 days before PYY(3–36) injections, and even longer habituation periods have been
reported (32), suggesting that some unaccounted strain or experimental differences need to be resolved before definitive conclusions can be made. It is also possible that the route of administration is part of the explanation for the variable results seen in the literature. Recently, several groups showed that intravenous administration of PYY(3–36) reduces food intake (12, 31). Although potent and reproducible results after intravenous administration of PYY(3–36) were reported, Scott et al. (31) failed to reproducibly inhibit food intake when PYY(3–36) was administered intraperitoneally.

Notwithstanding inconsistencies of acute anorectic effects of PYY(3–36) in rodents, the data from our chronic studies of subcutaneously administered PYY(3–36) clearly demonstrate that continuous administration of PYY(3–36) is an effective weight-control agent. The relatively short half-life of PYY(3–36) could partly explain the differences. In dogs, the metabolic half-life of PYY(3–36) is 110 min (29). In rats, peak plasma levels after an injection of PYY(3–36) were observed 15 min after injection, but the half-life was not reported (7). Although human PYY(3–36) levels rise sharply after a large test meal (4,500 kcal) and remain elevated for up to 6 h after the meal (3), PYY(3–36) levels decline to baseline within 30 min after PYY(3–36) infusion (7), suggesting that a single injection of PYY(3–36) does not accurately reflect the PYY(3–36) release pattern after a meal, which could be part of the explanation for the difficulty in consistently demonstrating anorectic effects of acute PYY(3–36) injections in rats and mice. Continuous intravenous infusions of PYY(3–36) for 90 min to rats potently suppresses food intake for up to 12 h (12), which is consistent with observations from humans (7).

![Energy expenditure and respiratory exchange ratio](image)

**Fig. 5.** Energy expenditure (i.e., heat) and respiratory exchange ratio [RER, i.e., CO₂ production ÷ O₂ consumption (V˙CO₂/V˙O₂)] measured at thermoneutrality (29°C) for 6 h in rats acclimatized to 29°C for 15 h. Data from the first 2 h of calorimetry (test habituation period) were excluded from analysis. Measurements were performed on days 14–17. Values are means ± SE.

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Table 3. Blood biochemistry on day 26

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>25 µg·kg⁻¹·day⁻¹</th>
<th>250 µg·kg⁻¹·day⁻¹</th>
<th>1,000 µg·kg⁻¹·day⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-glucose (mmol/l)</td>
<td>7.3±0.2</td>
<td>7.1±0.2</td>
<td>6.9±0.2</td>
<td>6.8±0.2</td>
</tr>
<tr>
<td>P-insulin (pmol/l)</td>
<td>380±48</td>
<td>293±40</td>
<td>289±43</td>
<td>258±43*</td>
</tr>
<tr>
<td>P-FFA, mmol/l</td>
<td>0.87±0.07</td>
<td>0.95±0.06</td>
<td>0.94±0.06</td>
<td>1.14±0.15</td>
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<tr>
<td>P-cholesterol, mmol/l</td>
<td>1.88±0.10</td>
<td>1.90±0.12</td>
<td>1.76±0.14</td>
<td>1.53±0.09*</td>
</tr>
<tr>
<td>P-TG, mmol/l</td>
<td>2.00±0.16</td>
<td>1.91±0.34</td>
<td>1.80±0.31</td>
<td>1.54±0.20</td>
</tr>
<tr>
<td>hPYY, pg/ml</td>
<td>221±78</td>
<td>315±73</td>
<td>4,662±295*</td>
<td>6,890±2,240*</td>
</tr>
</tbody>
</table>

Values are means ± SE. P, plasma; FFA, free fatty acid; TG, triglyceride; hPYY, human peptide YY. *P < 0.05 vs. vehicle (ANOVA followed by Fisher’s post hoc analysis).

![Changes in blood glucose and insulin](image)

**Fig. 6.** Changes in blood glucose (A) and insulin (B) after administration of glucose (2 g/kg po) at time 0. Values are means ± SE (n = 10). □, Vehicle; ■, 100 µg·kg⁻¹·day⁻¹ PYY(3–36); ▼, 300 µg·kg⁻¹·day⁻¹ PYY(3–36); ○, 1,000 µg·kg⁻¹·day⁻¹ PYY(3–36).
PYY(3–36) lowers body weight in diet-induced obesity

Table 4. AUC for glucose and insulin from OGTT and ISI from DID rat study

<table>
<thead>
<tr>
<th></th>
<th>AUC Glucose, mmol l⁻¹ min⁻¹</th>
<th>AUC Insulin, (pmol l⁻¹ min⁻¹) × 10⁻³</th>
<th>ISI × 10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1.153±0.71</td>
<td>71.4±7.1</td>
<td>24.37±2.88</td>
</tr>
<tr>
<td>25 µg·kg⁻¹·day⁻¹ PYY(3–36)</td>
<td>1.165±0.81</td>
<td>54.0±5.8*</td>
<td>36.15±6.64</td>
</tr>
<tr>
<td>250 µg·kg⁻¹·day⁻¹ PYY(3–36)</td>
<td>1.052±0.59</td>
<td>51.2±3.9†</td>
<td>38.21±6.35</td>
</tr>
<tr>
<td>1,000 µg·kg⁻¹·day⁻¹ PYY(3–36)</td>
<td>1.004±0.61</td>
<td>40.0±2.2‡</td>
<td>45.84±7.41*</td>
</tr>
</tbody>
</table>

Values are means ± SE. AUC, area under the curve; OGTT, oral glucose tolerance test; DID, diet-induced obese; ISI, insulin sensitivity index. For AUC glucose, baseline = 6 mmol/l; for AUC insulin, baseline = 0 pmol/l. Significant difference from vehicle (ANOVA followed by Fisher’s post hoc analysis): *P < 0.05; †P < 0.01; ‡P < 0.0001.

The underlying mechanism of PYY(3–36)-induced anorexia is not completely understood, but it has been proposed that circulating PYY(3–36) interacts with inhibitory presynaptic Y2 receptors located on neuropeptide Y neurons in the hypothalamic arcuate nucleus (7). However, Y2 receptors are also abundantly expressed in the dorsal vagal complex, as well as in the nodose ganglion (13). Recently, it was shown that PYY(3–36)-induced anorexia is attenuated when vagal afferent signaling is compromised (1, 20), but this finding was recently challenged by Halatchev and Cone (18), who showed a persistence of PYY(3–36) anorexia in mice with bilateral subdiaphragmatic vagotomy. These authors also showed that 15 and 50 µg/kg PYY(3–36) administered intraperitoneally caused conditioned taste aversion in mice and suggested that “These data raise the possibility that the primary mechanism by which PYY3–36 inhibits food intake in mice is through a short-term aversive response” (18). Although it is very difficult to demonstrate that the taste aversion elicited by PYY(3–36) is responsible for the anorexia, at least PYY(3–36)-induced anorexia has been demonstrated to be a Y2 receptor-mediated effect, because 1) PYY(3–36) does not inhibit food intake in Y2 receptor-knockout mice (7) and 2) PYY(3–36) anorexia can be blocked by the specific Y2 receptor antagonist BIIE0246 (31). The absence of nausea during PYY(3–36) infusions in humans (6, 7) and no signs of nausea when PYY(3–36) was administered to nonhuman primates (21, 27) support a specific anorectic effect. However, because PYY(3–36) has been reported to cause aversion in mice (18) and because relatively high doses of PYY(3–36) were used in the present study, we tested the ability of PYY(3–36) (250 and 1,000 µg/kg) to elicit a conditioned taste aversion. Also the possibility that reductions in overall locomotor activity could be responsible for PYY(3–36)-induced anorexia was tested. In a standard two-bottle taste aversion assay, PYY(3–36) was unable to elicit a conditioned taste aversion, whereas the aversion elicited by the control substance LiCl was robust. Interestingly, rather than a decrease in general locomotor activity, a slight but significant increase in locomotor activity was seen in rats treated with PYY(3–36). PYY(3–36) at 250 and 1,000 µg/kg increased locomotor activity to the same extent. Although it is difficult to extrapolate from an acute test session, it is possible that increased locomotor activity could contribute to the weight loss observed after chronic dosing at 250 and 1,000 µg·kg⁻¹·day⁻¹. The observations from the acute taste aversion study are consistent with the behavioral observations made throughout the chronic dosing studies: 1) neither mice nor rats showed signs of abnormal behavior during the PYY(3–36) infusion period, and 2) food intake returned to baseline values ≤7 days into the infusion period, suggesting that the animals were not chronically nauseated. The exact reason for the discrepancy between our results and those reported by Halatchev and Cone remains to be elucidated, but in both

Fig. 7. A: saccharin preference ratio [saccharin intake/saccharin intake + tap water intake] × 100% after a single intraperitoneal injection of PYY(3–36) or LiCl during the first test session (72–84 h after injection). Only LiCl significantly reduced saccharin preference ratio. B: general locomotor activity for 17 h after PYY(3–36) and LiCl administration. Note small increase in general locomotor activity in rats treated with PYY(3–36) and small decrease in rats treated with LiCl. Values are means ± SE (n = 10). □, Vehicle; ▼, 250 µg·kg⁻¹·day⁻¹ PYY(3–36); ●, 1,000 µg·kg⁻¹·day⁻¹ PYY(3–36); *80 mg/kg LiCl. Significant difference between vehicle and PYY(3–36) or LiCl treatment: *P < 0.05 (250 µg/kg PYY vs. vehicle); †P < 0.05 (1,000 µg/kg PYY vs. vehicle).
species (mice and rats), the dose and taste aversion paradigm differed considerably.

In conclusion, the present results support the notion that PYY(3–36) is involved in appetite regulation. Although it could be argued that the relatively high doses of PYY(3–36) used in the present study [giving rise to pharmacological levels of circulating PYY(3–36)] do not unravel the role of endogenous PYY(3–36) in appetite and body weight regulation, the results demonstrate that chronic Y2 agonism is a powerful way to lower body weight and, hence, suggest that Y2 receptor agonists could be used for the treatment of obesity. Importantly, the results demonstrate long-lasting effects on body weight in two different rodent models of DIO with accompanying improvements in glucose homeostasis.

ACKNOWLEDGMENTS

We thank Jette Jørgensen for expert technical assistance.

REFERENCES


