Original Article

Peptide $YY_{(3–36)}$ Inhibits Morning, but Not Evening, Food Intake and Decreases Body Weight in Rhesus Macaques

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Peptide $YY_{(3–36)}$ [$PYY_{(3–36)}$] is a hormone that is released after meal ingestion that is currently being investigated for the treatment of obesity; however, there are conflicting reports of the effects of $PYY_{(3–36)}$ on energy balance in rodent models. To shed light on this controversy, we studied the effect of $PYY_{(3–36)}$ on food intake and body weight in a nonhuman primate. Intravenous $PYY_{(3–36)}$ infusions before a morning meal transiently suppressed the rate of food intake but did not suppress the evening meal or 24-h intake. Twice-daily or continuous intravenous $PYY_{(3–36)}$ infusions to supraphysiological levels (levels that exceeded normal physiological levels) again suppressed the rate of feeding for the morning but not the evening meal. Twice-daily intravenous $PYY_{(3–36)}$ infusions for 2 weeks significantly decreased body weight in all test animals (average weight loss 1.9%) without changing insulin response to glucose infusion. These results show that endogenous $PYY_{(3–36)}$ may alter morning but not evening meal intake, and supraphysiological doses are required for effective suppression of food intake. *Diabetes* 54: 3198–3204, 2005

The rising prevalence of obesity in the U.S. and other countries (1,2) is linked to increases in the incidence of obesity-related diseases (diabetes, cardiovascular disease, hypertension, and cancer), elevated health care costs, and reduced quality of life (3,4). The success of pharmacological intervention to reverse trends in obesity demographics depends on a better understanding of the physiology of appetite and body weight regulation. Because of their involvement in the regulation of energy homeostasis, hypothalamic and brainstem systems are major targets for pharmacological treatment of obesity (5,6).

The arcuate nucleus of the hypothalamus (ARH) contains two cell types that act antagonistically to regulate energy intake and expenditure: activation of cells that express proopiomelanocortin produces anorectic effects, whereas activation of cells that produce neuropeptide Y (NPY) elicits feeding and energy conservation (7). Ingestion of nutrients causes L-cells in the gastrointestinal tract to release $PYY_{(1–36)}$, which is an endogenous ligand for several NPY receptors (Y1, Y2, and Y5) (8). However, a cleavage product of $PYY_{(1–36)}$, $PYY_{(3–36)}$, is relatively selective for the NPY Y2 receptor (9). The NPY Y2 receptor is expressed in the ARH and other sites and is the dominant inhibitory autoreceptor on NPY neurons (10,11). Evidence of a nonsaturable transport mechanism for $PYY_{(3–36)}$ across the blood-brain barrier (12), coupled with recent data by Riediger et al. (13) that physiological doses of $PYY_{(1–36)}$ induced c-Fos in the ARH but not the area postrema (in the hindbrain), support the hypothesis that circulating $PYY_{(3–36)}$ is thought to suppress appetite through inhibition of ARH NPY neurons, but this does not exclude other possible sites of action.

Peripheral $PYY_{(3–36)}$ administration reduces food intake in humans and rodents (14–16). Although the effects of $PYY_{(3–36)}$ on gastric emptying are reproducible (17–19), the effectiveness of $PYY_{(3–36)}$ at inhibiting feeding in rodents is inconsistent. Recently published results demonstrate that intramuscular $PYY_{(3–36)}$ injection effectively delays gastric emptying and modestly reduces food intake in rhesus monkeys (20), yet plasma $PYY_{(3–36)}$ levels that produced this effect were not reported, and the effect of continuous $PYY_{(3–36)}$ treatment on body weight remains to be determined. Therefore, we addressed the efficacy of physiological and pharmacological doses of $PYY_{(3–36)}$ at reducing food intake and body weight in the rhesus monkey.

RESEARCH DESIGN AND METHODS

Adult male rhesus macaques (*Macaca mulatta*) were singly housed at the Oregon National Primate Research Center (ONPRC) in an environment controlled for humidity, temperature, and lighting (illuminated between 7:00 A.M. and 7:00 P.M.). Nine animals between 10 and 18 years old and between 8 and 23 kg body wt were studied. All procedures were approved by the ONPRC Institutional Animal Care and Use Committee. Experimental group size was five or more monkeys unless otherwise noted.

Animals were fed low-fat monkey diet (#5047, jumbo size; Purina, St. Louis, MO) twice daily. Generally, animals were fed 12 biscuits at 10:00 A.M. and again at 3:00 P.M. Biscuits weighed ~16.5 g and had an energy density of 3.11 kcal/g (25% protein, 5% fat, 6.5% fiber, 6% ash, and 57.5% carbohydrate). A high-fat diet consisting of semi-solid cubes weighing ~60 g with an energy density of 3.9 kcal/g (20% protein, 35% fat, and 45% carbohydrate) was provided as noted during specific experiments. Macronutrient composition of the high-fat diet was designed to mimic a typical North American diet (21). Drinking water was continuously available via pressurized drinking spouts.

Surgery and instrumentation. Two chronically implanted catheters placed into separate subclavian, femoral, or jugular veins of each animal allowed for simultaneous infusion of $PYY_{(3–36)}$ and collection of blood samples. Catheters remained in place for the duration of the experiments and were remotely accessible in an adjacent sampling room. Monkeys wore fitted nylon vests that

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ARH, arcuate nucleus of the hypothalamus; IVGTT, intravenous glucose tolerance test; NPY, neuropeptide Y; ONPRC, Oregon National Primate Research Center; $PYY_{(3–36)}$, peptide $YY_{(3–36)}$.

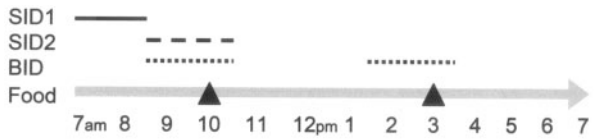

FIG. 1. Feeding and once- and twice-daily $PYY_{(3–36)}$ infusion schedule. Vehicle and low-dose (0.3 pmol · kg^{-1} · min^{-1}) once-daily infusions (SID1, solid line) began at 7:00 A.M. and ended at 8:30 A.M. High-dose (0.8 pmol · kg^{-1} · min^{-1}) once-daily infusions (SID2, dashed line) began at 8:30 A.M. and ended at 10:30 A.M. Morning twice-daily infusions (BID, dotted line) began at 8:30 A.M. and ended at 10:30 A.M.; evening twice-daily infusions began at 1:30 P.M. and ended at 3:30 P.M. Animals were fed, and previously unconsumed food was removed at 10:00 A.M. and 3:00 P.M. (gray arrow). ▲, food change.

were connected to 36-inch flexible metal tethers, which were fastened to swivels mounted to the tops of cages (22). This catheter protection system allowed for blood sampling and intravenous infusions without sedating or disturbing animals and allowed animals free range of movement within their cages at all times. Physiological saline drip (5 ml/h; Baxter Healthcare, Deerfield, IL) containing heparin sodium (4 units/ml) maintained catheter patency. Catheters were checked daily for patency, and animals were sedated weekly with Ketaset (ketamine hydrochloride, 10 mg/kg i.m.) to record body weight and inspect catheter systems.

Blood collection and assay. Blood collections were performed remotely via intravenous catheters. Blood was drawn into syringes prerinsed with heparin (heparin sodium, 10,000 units/ml), transferred to test tubes containing peptidase inhibitor (aprotinin, 0.6 trypsin inhibiting units/ml blood; Sigma Chemical), and centrifuged at 1,600*g* for 15 min at 4°C. Plasma was transferred into polypropylene freezer vials and stored frozen at −80°C until assayed.

All samples were assayed in duplicate. Total PYY immunoreactivity was measured with a sensitive radioimmunoassay that detected both the cleaved form ($PYY_{3–36}$) and the full-length hormone ($PYY_{1–36}$); as described previously, all the results from our assays reflect total plasma PYY (15). Briefly, the assay was validated for monkey using serial dilutions of a pool of samples of high $PYY_{(3–36)}$ values and performed in a total volume of 350 μl of 0.06 mol/l phosphate buffer, pH 7.3, containing 0.3% BSA. Samples were incubated for 3 days at 4°C before separation of free and antibody-bound label by goat anti-rabbit IgG serum. One hundred microliters of unextracted heparinized plasma was assayed. The lowest detectable level was 3.2 pg/tube. Intra-assay variation was determined at concentrations of 100 and 400 pg/ml and was 6.3 and 6.6%, respectively. Interassay variation was 7.2 and 8.1% for the range of values measured.

Several different sampling schedules were used to determine endogenous release of total PYY [both $PYY_{(1–36)}$ and $PYY_{(3–36)}$]. For one study, samples were drawn hourly before, during, and after animals ate normal meals of low-fat diet (n = 4), high-fat diet (n = 2), or after fasting (n = 2). In a subsequent study, blood samples were taken 15, 30, 45, and 60 min after a 3:00 P.M. meal and every 30 min thereafter until 9:00 P.M., and two baseline blood samples were taken 30 and 60 min before feeding of a low-fat (n = 6) or high-fat (n = 6) meal. A final study involved blood sampling either at baseline (3:00 P.M. after a morning fast) or at 8:00 P.M. after ad libitum consumption of high-fat diet (n = 6), low-fat diet (n = 6), or fasting (n = 6).

$PYY_{(3–36)}$ test infusions. Synthetic human $PYY_{(3–36)}$ was obtained from Phoenix Pharmaceuticals (Belmont, CA). The (3–36) fragment of PYY was used for all infusion tests. Vehicle for $PYY_{(3–36)}$ infusions was physiological saline containing 1% human serum albumin (Albuminar-25; Aventis Behring, Kankakee, IL).

Preliminary infusion studies established appropriate doses for approximating normal total PYY in fasted animals. Three separate 60-min infusions of $PYY_{(3–36)}$ in ascending concentrations of 0.8, 2.4, and 4.8 pmol · kg^{-1} · min^{-1} were separated by 30-min saline washout periods (n = 3). A second set of test infusions with lower doses (0.2, 0.3, and 0.4 pmol · kg^{-1} · min^{-1}) allowed accurate approximation of total postprandial PYY (n = 3). Animals were closely monitored to detect any adverse reactions to $PYY_{(3–36)}$ dosing. Intermittent blood sampling determined the achieved circulating total PYY levels and guided the selection of doses for initial $PYY_{(3–36)}$ infusion experiments.

Once-daily infusions. Three dosing protocols were used: once-daily, twice-daily, and continuous infusion (see Fig. 1). The once-daily dosing protocol (n = 5–6) was used to test the hypothesis that elevation of circulating $PYY_{(3–36)}$ levels before and during the morning meal decreases food intake and body weight. Seven days of vehicle infusion were followed by 7 consecutive days of $PYY_{(3–36)}$ infusion at 0.3 pmol · kg^{-1} · min^{-1} (because this dose mimics total postprandial PYY levels, as determined by assay as described above) and 2 days at 0.8 pmol · kg^{-1} · min^{-1}. Vehicle and low-dose (0.3 pmol · kg^{-1} · min^{-1}) $PYY_{(3–36)}$ infusions began at 7:00 A.M. and ended at 8:30 A.M., and the higher $PYY_{(3–36)}$ dose (0.8 pmol · kg^{-1} · min^{-1}) began at 8:30 A.M. and ended at 10:30 A.M. Animals were fed at 10:00 A.M. throughout the duration of the study. Detailed feeding pattern measurements were made on the last 2 days of vehicle infusion and the first 2 days of 0.3 pmol · kg^{-1} · min^{-1} and 0.8 pmol · kg^{-1} · min^{-1} $PYY_{(3–36)}$ infusions. Total 24-h food intake was measured daily. Two blood samples were taken 15 min before and 15 min after the start of each infusion with the exception of the 1st day of the low-dose $PYY_{(3–36)}$ infusion, when additional blood samples were taken at 30, 60, 90, 120, and 240 min after the infusion. During the high-dose $PYY_{(3–36)}$ infusions, blood samples were taken 15 min before and 20 min after meal presentation.

Twice-daily infusions. The second set of experiments tested the hypothesis that daily $PYY_{(3–36)}$ infusion before and during morning and evening meal onset suppresses food intake and leads to body weight loss (n = 5–6). Infusions occurred between 8:30 and 10:30 A.M. and 1:30 and 3:30 P.M., daily. Seven days of vehicle infusions preceded and followed $PYY_{(3–36)}$ infusions. $PYY_{(3–36)}$ (0.8 pmol · kg^{-1} · min^{-1}) was infused for 5 days, followed by 11 days of 1.6 pmol · kg^{-1} · min^{-1}. Animals were weighed before and after the vehicle treatment week, after the 5th day of the low $PYY_{(3–36)}$ dose (PYY1), and again after the higher $PYY_{(3–36)}$ dose (PYY2). Total daily food intake was recorded throughout the twice-daily infusion protocol, and detailed feeding patterns were recorded on 7th day of initial vehicle infusion, the 1st day of PYY1, days 1 and 5 of PYY2, and days 1 and 7 of the after treatment vehicle period. Blood samples were taken 15 min before and after meals and 30 min after the end of $PYY_{(3–36)}$ infusions.

Continuous infusion. Three days of $PYY_{(3–36)}$ treatment (1.6 pmol · kg^{-1} · min^{-1}; n = 3) were preceded and followed by 3 days of continuous vehicle infusion. Detailed measurements of feeding patterns were made on each day of treatment. Blood samples were drawn 15 min before both the morning and evening meals to assess total plasma PYY.

Behavioral analysis. Animals (n = 5) were videotaped for 6 hours on a single day of vehicle and on a single day of $PYY_{(3–36)}$ infusion (1.6 pmol · kg^{-1} · min^{-1} for 120 min) during the twice-daily infusion protocol to assess effects of $PYY_{(3–36)}$ on general behavior and to determine whether appetite-suppressing effects of $PYY_{(3–36)}$ were linked to sickness or malaise. Video cameras were placed on tripods in front of cages for several days before treatment to accustom animals to the presence of recording equipment. Behavioral analysis of focal observations was performed with a videotape player that was synchronized with a computer via The Observer 3.0 Software (Noldus Technologies). An observer blind to treatment scored the frequencies and durations of a battery of behaviors chosen to maximize detection of illness. Scored behaviors included drinking, eating food, touching food, standing, lying on side of body, locomotion, grooming, yawning, napping, salivation, vomiting, and stereotypical behavior. Frequency data are presented as mean occurrences per 6-h observation period ± SEM. Durations are presented in cumulative seconds of duration ± SEM during the 6-h observation period.

Intravenous glucose tolerance tests. Intravenous glucose tolerance tests (IVGTTs) (n = 6) were performed by measuring blood glucose clearance after an intravenous bolus infusion of a sterile 50% dextrose solution (600 mg/kg). IVGTTs were performed before any vehicle testing, after 1 week of vehicle infusions, and again after twice-daily $PYY_{(3–36)}$ treatment. Animals were not fed on the morning of IVGTTs. Blood glucose was measured immediately in whole blood with an Accu-chek Advantage blood glucose meter (Roche Diagnostics). Plasma was assayed for insulin using a Roche Diagnostics Elecysis 2010 analytical instrument by the ONPRC/OHSU Endocrine Services Laboratory. The interassay percentage of coefficient of variation for this assay was 3.5%. A subset of plasma samples from the IVGTT (n = 3) was assayed for total plasma PYY levels.

Analysis. Statistical Software package SPSS 11.5 for Windows (SPSS) was used for all statistical calculations to evaluate the effect of $PYY_{(3–36)}$ dose on feeding and body weight. To allow for combined analysis of multiple treatment days, doses, and time points in the same animals, the SPSS Linear Mixed Model was used for analyses unless specified otherwise. This model is often used for unbalanced datasets with nonuniform design and/or missing subjects because it estimates the marginal means for each dose and treatment regimen (we derived the degrees of freedom from these numbers). Like treatments (doses) from different test days and different times were collapsed within the model and used in the analysis to generate statistics for the overall effect of dose on the appropriate dependent variable. We did not collapse data across treatment days because of the small sample size inherent in monkey studies and the use of a linear mixed model. This increases the degrees of freedom but captures other variability inherent in the test protocols, and we believe it is the best method of analysis for this data. There was not enough statistical power to draw meaningful conclusions about the day-to-day repeatability and strength of the effect during the once- and twice-daily treatment protocols. Where appropriate, estimated marginal means generated by the statistical model were used to calculate an overall difference between doses for an experiment, and post hoc comparisons (Bonferroni adjusted) were used to

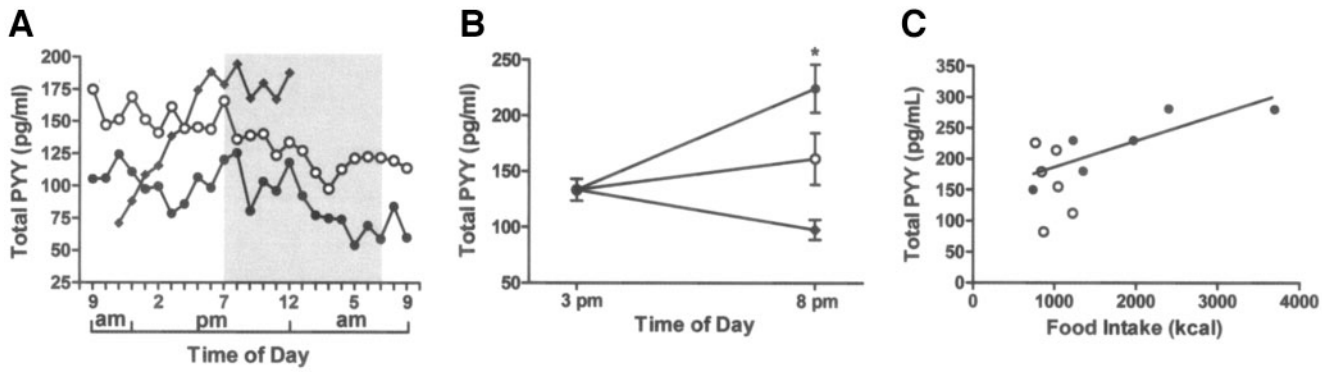

FIG. 2. Total plasma PYY in monkeys. *A*: Total PYY for animals on a low-fat diet (○; $n = 4$), high-fat diet (●; $n = 2$), or fasted (diamonds; $n = 2$). *B*: Total PYY after spontaneous intake of low-fat diet ($n = 6$) or high-fat diet ($n = 6$) or in fasted animals ($n = 6$) differed significantly in all three conditions at 8:00 P.M. ($n = 6$, $P < 0.05$). *C*: Intake of high-fat diet (●; $n = 6$) but not low-fat diet (○; $n = 6$) was positively correlated with total PYY measured 5 h after meal presentation ($r^2 = 0.71$, $P = 0.03$).

compare effects between individual doses at specific time points. Body weight change during vehicle infusion was compared with body weight change during $PYY_{(3–36)}$ treatment using paired t test. Graphs depict means of data ± 1 SEM. Data from days with the same treatment doses are displayed as means in graphs. The effect of $PYY_{(3–36)}$ infusion on scored behaviors was statistically evaluated using paired sample t tests.

RESULTS

Total basal PYY levels in monkeys. Total endogenous PYY ranged between 100 and 175 pg/ml and gradually declined overnight (Fig. 2*A*). Total PYY was positively correlated with intake of high-fat meal ($r^2 = 0.71$, $P = 0.03$); spontaneous intake of low-fat diet caused no significant change in total PYY; and fasting caused gradual decline with a nadir of ~50 pg/ml overnight (Fig. 2*A*–*C*). The between-subjects variation in total PYY in response to spontaneous ingestion of low-fat diet ranged between 75 and 225 pg/ml (Fig. 2*C*).

Test infusions of $PYY_{(3–36)}$ to mimic physiological blood levels. Total PYY after test infusions (0.2, 0.3, and 0.4 pmol · kg^{-1} · min^{-1}) was about one to three times greater than circulating endogenous postprandial levels (Fig. 3*A*). Infusions of 0.8, 2.4, and 4.8 pmol · kg^{-1} · min^{-1} produced peak total PYY ranging from 500 pg/ml to >5,000 pg/ml (assay upper limit, Fig. 3*B*). Careful observation of animals during test infusions did not reveal any indication of illness or altered behavior (see detailed behavioral analysis; Table 1). An assay for plasma cortisol also revealed no changes in cortisol concentrations during $PYY_{(3–36)}$ infusions.

Once-daily infusions. $PYY_{(3–36)}$ infusion before and during the morning meal at doses that approximated endogenous postprandial levels, and at supraphysiological levels, suppressed acute food intake during that meal. Once-daily $PYY_{(3–36)}$ infusion dose-dependently suppressed the rate of eating (Fig. 4*A*; dose by time interaction; $F_{(14, 241.986)} = 2.255$, $P = 0.007$), but total consumption for the morning meal was not affected ($P \geq 0.369$). Morning $PYY_{(3–36)}$ infusion did not affect rate of eating or total food consumption during the evening meal (Fig. 4*B*; dose-by-time interaction; $F_{(8, 212.110)} = 0.721$, $P = 0.665$) and had no effect of on total 24-h food intake (Fig. 4*C*; tests for effect of dose based on estimated marginal means: $F_{(2, 26.218)} = 0.843$, $P = 0.442$). The time course for total daily food intake is shown in Fig. 4*D*.

Twice-daily infusions. Infusions of $PYY_{(3–36)}$ that increased total PYY levels two to five times physiological levels before and during the morning (Fig. 5*A*) and evening meals (Fig. 5*B*) reduced the initial rate of eating only during the morning meal (Fig. 5*A*; dose-by-time interaction, morning meal: $F_{(21, 481)} = 22.642$ $P < 0.001$) and produced a mild suppression in 24-h food intake that was not significant ($F_{(2, 56.086)} = 2.997$, $P = 0.058$; Fig. 5*C*). Multiple pairwise comparisons of the effect of $PYY_{(3–36)}$ at individual time points during the morning meal showed significant reductions in intake with the 1.6 pmol · kg^{-1} · min^{-1} dose at 11:00 A.M. but not at the end of the morning meal or during the evening meal (Fig. 5*A* and *B*). Post hoc comparison of 1.6 pmol · kg^{-1} · min^{-1} versus vehicle doses revealed marginal significance ($P = 0.052$). Daily food intake was generally inhibited during the entire twice-daily $PYY_{(3–36)}$ infusion period (Fig. 5*D*). There may be diminished effectiveness of the drug at the end of the $PYY_{(3–36)}$ infusion period, although we did not have the statistical

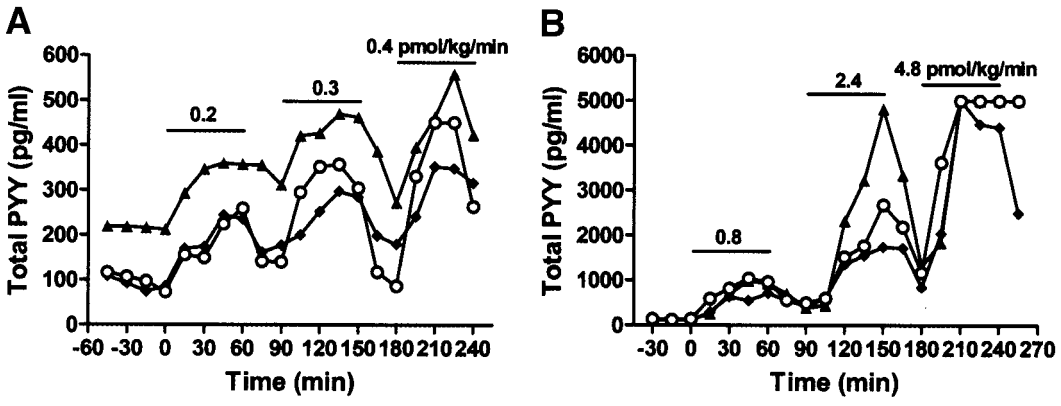

FIG. 3. Total plasma PYY during test infusions. *A*: $PYY_{(3–36)}$ infusions that modestly raised total PYY above normal physiological levels ($n = 3$). *B*: $PYY_{(3–36)}$ infusions in which total PYY greatly exceeded physiological levels ($n = 3$). Different symbols represent individual animals.

TABLE 1
Frequency and duration of behaviors

	Frequency (events/6 h)		Duration (s)	
	Vehicle	PYY	Vehicle	PYY
Drinking	6.4 ± 1.3	5.8 ± 2.4	114.3 ± 15.7	78.7 ± 24.4
Eating biscuit	15.2 ± 1.4	17.4 ± 6.4	1,739.6 ± 470.0	1,384.3 ± 460.5
Touching biscuit	7.8 ± 2.3	17.0 ± 5.2*	89.1 ± 17.5	150.9 ± 87.6
Standing	48.2 ± 10.4	38.0 ± 13.1	352.0 ± 113.0	295.8 ± 126.1
Lying on side	6.8 ± 5.1	7.2 ± 4.4	378.8 ± 284.4	487.9 ± 248.7
Locomotion	23.0 ± 8.0	15.8 ± 4.9	69.1 ± 25.8	107.5 ± 70.8
Grooming	33.2 ± 16.3	28.4 ± 15.7	1,100.6 ± 605.8	1,033.8 ± 738.0
Yawning	45.8 ± 9.8	44.8 ± 5.2	NA	NA
Napping	0.0 ± 0.0	2.0 ± 1.4	0.0 ± 0.0	209.2 ± 189.8

Data are means ± SEM. *$P < 0.05$ vs. vehicle.

power to analyze the data for day to day differences in effect of $PYY_{(3–36)}$.

The twice-daily $PYY_{(3–36)}$ infusion caused a 1.9 ± 0.7% body wt loss (Fig. 5*E*; $P = 0.017$) that persisted through the second $PYY_{(3–36)}$ dosing period (Fig. 5*F*). There was no additional weight loss, and body weight returned to baseline after cessation of treatment.

Behavioral analysis. Analysis of videotapes revealed no nonspecific effects of the 1.6 pmol · kg^{-1} · min^{-1} $PYY_{(3–36)}$ infusion on behavior (Table 1). There was a significant increase in the frequency ($P = 0.028$), but not duration, of touching biscuits. There were no statistical differences in grooming, locomotion, or napping and in no cases did animals display salivation or drooling, nor did animals exhibit general malaise. One animal appeared to regurgitate into its cheek pouches several times during the $PYY_{(3–36)}$ infusion experiment (such regurgitation is a normal occurrence in some animals), but no overt vomiting was observed. No regurgitation or vomiting was observed in any other animals.

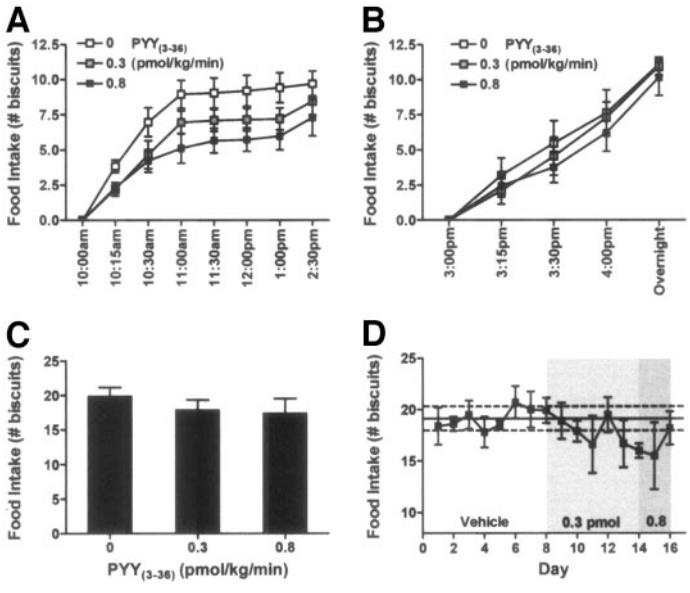

FIG. 4. Effect of once-daily $PYY_{(3–36)}$ infusion on food intake. *A*: Once-daily $PYY_{(3–36)}$ infusion suppressed morning meal intake [$n = 5–6$, dose-by-time interaction; $F_{(14, 241.986)} = 2.255$, $P = 0.007$], but total meal intake was not affected for morning (*A*), evening (*B*), or 24-h (*C*) total. Data from the last 2 days of vehicle infusion and the first 2 days of each of the $PYY_{(3–36)}$ doses are combined in *A* and *B*, and the complete dataset from the 7 days of vehicle, 7 days of 0.3 pmol · kg^{-1} · min^{-1}, and 2 days of 0.8 pmol · kg^{-1} · min^{-1} is plotted in *C*. Daily food intake is plotted in *D* (solid line, average baseline intake ± SEM).

IVGTT. Repeated twice-daily $PYY_{(3–36)}$ infusion had no effect on glucose clearance or insulin response profiles during IVGTTs. There was no effect of supraphysiological elevation of blood glucose levels during IVGTT on total circulating PYY (data not shown).

Continuous infusions. Continuous 24-h $PYY_{(3–36)}$ infusion reduced food intake during the morning but not the evening meal (Fig. 6*A* and *B*; $F_{(1, 85.772)} = 18.356$, $P < 0.001$, $F_{(1, 86.183)} = 1.077$, $P = 0.302$, respectively; $n = 3$). Multiple comparisons of the effect of $PYY_{(3–36)}$ dose on intake at specific time points during the morning meal revealed significant differences at 11:00, 12:00, and 2:30 P.M. (Fig. 6*A*). Continuous $PYY_{(3–36)}$ infusion suppressed morning but not evening food intake (Fig. 6*C*). The decrease in morning food intake caused by $PYY_{(3–36)}$ infusion also decreased 24-h food intake in all animals (Fig. 6*D*; $F_{(1, 22)} = 9.746$, $P = 0.005$). Continuous $PYY_{(3–36)}$ infusion appeared to inhibit food intake each day of $PYY_{(3–36)}$ infusion, but because of the small sample size, we were unable to make comparisons across time (Fig. 6*E*).

Total plasma PYY during experiments. Total immunoreactive plasma PYY increased during $PYY_{(3–36)}$ infusion. Furthermore, total PYY rose in proportion to increasing $PYY_{(3–36)}$ infusion doses. At the end of the 0.3 pmol · kg^{-1} · min^{-1} s.i.d. infusions, average total PYY reached levels of ~250 pg/ml—close to maximum observed endogenous levels (Fig. 7*A*). Total PYY levels increased to ~450 pg/ml after 0.8 pmol · kg^{-1} · min^{-1} s.i.d. infusions. Total PYY during meal time in the twice-daily infusion protocol are shown in Fig. 7*B*. Because of an infusion problem, the 1st day of twice-daily $PYY_{(3–36)}$ dosing was 0.6 pmol · kg^{-1} · min^{-1} (instead of 0.8 pmol · kg^{-1} · min^{-1}), and this was reflected in total PYY levels. Total PYY achieved after infusion of 0.8 pmol · kg^{-1} · min^{-1} was ~500–800 pg/ml and increased to between 1,000 and 2,000 pg/ml for the 1.6 pmol · kg^{-1} · min^{-1} b.i.d. infusions. Total PYY decreased to about one-half of peak values 30 min after the infusions ended and quickly returned to baseline. Continuous infusion of $PYY_{(3–36)}$ at 1.6 pmol · kg^{-1} · min^{-1} caused total PYY levels to peak in excess of 2,000 pg/ml (Fig. 7*C*). This result indicates that our assay detected increases in total PYY that were likely due to elevated plasma $PYY_{(3–36)}$ from infusion, and our behavioral data are also primarily caused by action of $PYY_{(3–36)}$.

DISCUSSION

This study sheds light on previous challenges in reproducing findings that $PYY_{(3–36)}$ significantly decreases food intake (17–19). The data presented here demonstrate that,

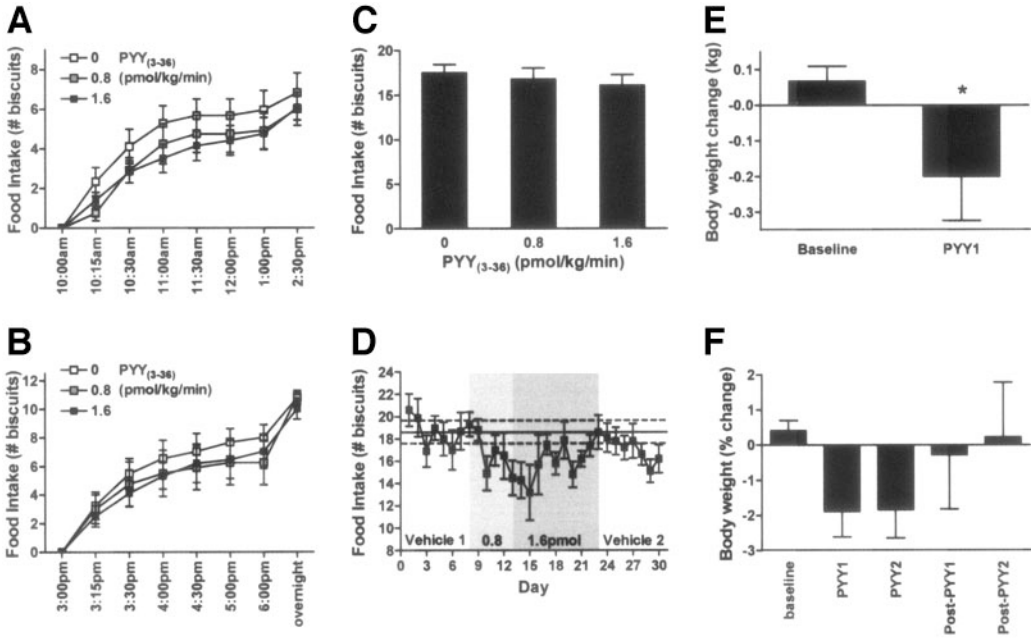

FIG. 5. Effect of twice-daily $PYY_{(3–36)}$ infusion on food intake and body weight. Twice-daily $PYY_{(3–36)}$ infusion reduced initial rate of eating during the morning (*A*) [dose-by-time interaction, $n = 5–6$, $F_{(21, 481)} = 22.642$, $P < 0.001$] but not the evening meal (*B*). *C*: Suppression of 24-h intake was marginally significant ($P = 0.052$). *D*: Daily food intake (solid line, average intake for vehicle 1 ± SEM). *E*: Body weight decreased during the first $PYY_{(3–36)}$ treatment (PYY1: 0.8 pmol · kg^{-1} · min^{-1}; $P = 0.017$). *F*: Weight loss was sustained through the second $PYY_{(3–36)}$ treatment (PYY2, 1.6 pmol · kg^{-1} · min^{-1}) and returned to pretreatment levels after cessation of $PYY_{(3–36)}$ infusions.

in this model, low doses of $PYY_{(3–36)}$ that mimic physiological levels do not significantly reduce food intake—only sustained supraphysiological PYY levels effectively decreased morning but not evening food intake regardless of infusion protocol. This is in contrast to the long-lasting effect of $PYY_{(3–36)}$ infusion in humans consuming a buffet meal in the middle of the day, who reported reduced appetite long after infusion cessation (15). In the case of once-daily infusions, the lack of an inhibitory effect on evening food intake is not surprising because elevated total morning PYY quickly returns to baseline after infusion. However, the finding that twice-daily and continuous infusions were only effective at suppressing morning meal intake is perplexing and requires further analysis.

One explanation is that decreased food intake in the morning increased hunger during the evening meal, superseding the minor suppressive effect of $PYY_{(3–36)}$. However, this idea does not account for the fact that animals in the continuous infusion protocol consistently reduced morning intake but did not compensate for the caloric deficiency by increasing their evening meal intake. Alternatively, Y2 receptors might desensitize after activation by $PYY_{(3–36)}$ in the morning, attenuating the effect of equal $PYY_{(3–36)}$ concentrations in the evening. However, this possibility is also inconsistent with our results from the continuous $PYY_{(3–36)}$ infusion protocol, in which animals consistently decreased their morning food intake, despite high total PYY throughout the entire day and night. It is also possible that priming by other signals (like cortisol) regulates sensitivity to $PYY_{(3–36)}$. Because circulating cortisol is higher in the morning, this hormone might prime the ARH or other brain areas to the anorexigenic effects of $PYY_{(3–36)}$, although much more extensive studies would be needed to explore this possibility. We feel that a fourth, and very likely, explanation is that because monkeys generally show a greater orexigenic drive in the afternoon compared with the morning (23), detection of a small anorexigenic effect in the afternoon is more difficult. Therefore, a mild anorexic signal, such as $PYY_{(3–36)}$, would be more easily detected in the morning when orexigenic drive is lower.

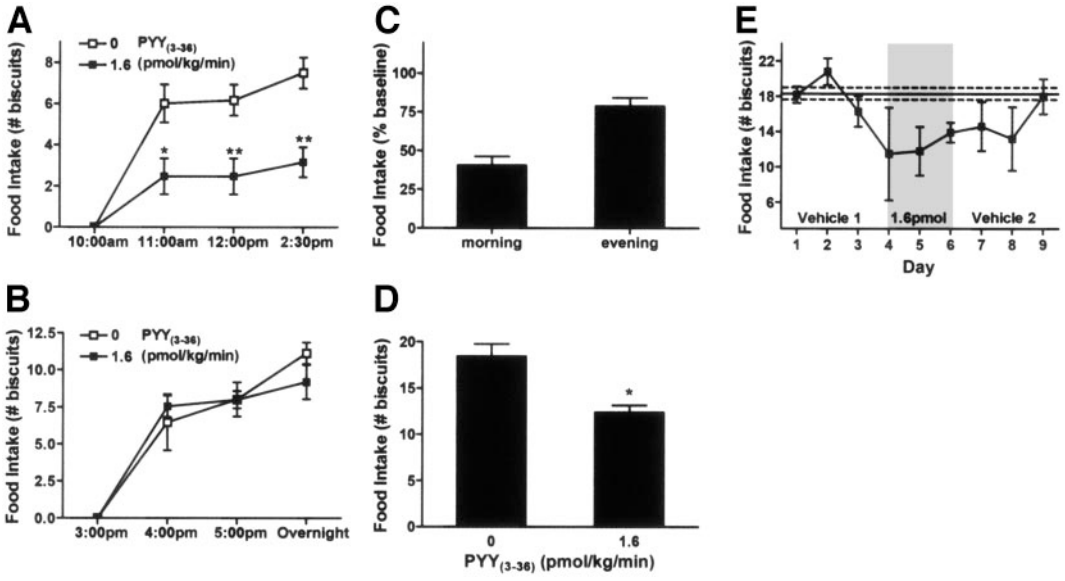

FIG. 6. Effect of continuous $PYY_{(3–36)}$ infusion on food intake. Continuous infusion of 1.6 pmol · kg^{-1} · min^{-1} $PYY_{(3–36)}$ suppressed morning intake (*A*) ($P < 0.001$, $n = 3$) but not evening intake (*B*) ($P = 0.302$, $n = 2$). *C*: Continuous infusion persistently reduced morning but not evening meal. *D*: The reduction in morning food intake affected total 24-h intake ($P = 0.005$). *E*: Daily food intake before, during, and after continuous $PYY_{(3–36)}$ infusion (solid line, average intake for vehicle 1 ± SEM).

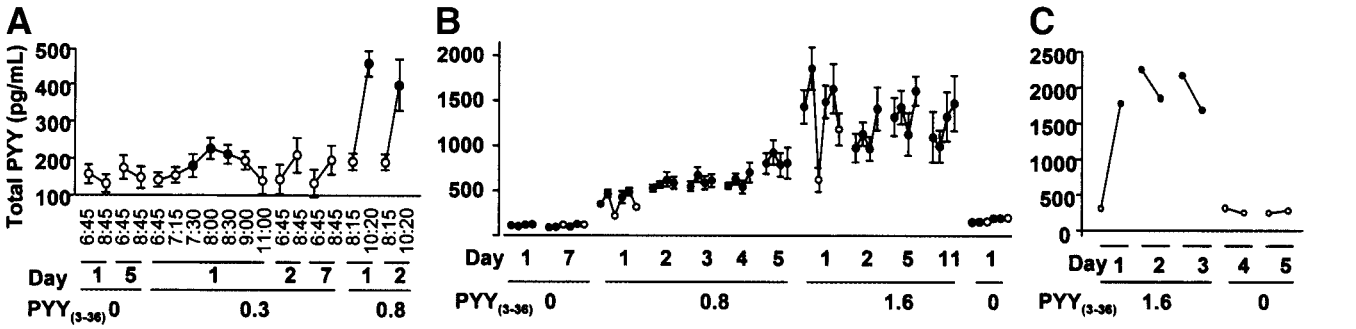

FIG. 7. Total plasma PYY during $PYY_{(3–36)}$ infusions. Total PYY (pg/ml) during once-daily (*A*), twice-daily (*B*), and continuous (*C*) infusion protocols reflects $PYY_{(3–36)}$ infusion dose. *A*: Infusion dose (pmol · kg^{-1} · min^{-1}), day of treatment, and time of sampling are shown on the abscissa [○, before $PYY_{(3–36)}$ infusion; ●, after $PYY_{(3–36)}$ infusion]. *B*: Plasma samples 15 min before and after both the morning and evening meals (●) or 30 min after the end of $PYY_{(3–36)}$ infusions (○). *C*: Total PYY during vehicle or continuous $PYY_{(3–36)}$ infusion. Samples were taken 15 min before each meal. ●, samples taken during $PYY_{(3–36)}$ infusion; ○, samples taken during vehicle infusion. Data represent mean of all animals.

In addition to satiety, stress and illness are powerful regulators of food intake. We performed a radioimmunoassay for plasma cortisol (enhanced release of this hormone is associated with heightened stress) and found no change in plasma cortisol levels between vehicle and $PYY_{(3–36)}$ infusions that ranged from 0.2 to 4.8 pmol · kg^{-1} · min^{-1} (data not shown). So although it is not likely that $PYY_{(3–36)}$ reduces food intake by increasing stress, $PYY_{(3–36)}$ might decrease appetite by inducing illness. For example, $PYY_{(3–36)}$ could reduce food intake by activating chemosensitive hindbrain regions that mediate emesis (Y2 receptors are present in the rodent hindbrain [24,25]), although no direct evidence supports this claim. Our behavioral analysis showed that increased frequency of touching food after $PYY_{(3–36)}$ treatment correlated with a nonsignificant decrease in the duration of eating. Although these data suggest an interest in food that is not actualized by consumption, the possibility that this behavior is an indication of simultaneous hunger and illness is inconsistent with the notable absence of other behavioral indicators of illness. Formal behavioral analysis for several indicators of sickness in monkeys found no differences between drug and vehicle in all monkeys but one, even when total PYY was 20 times normal physiological levels. More notably, clinical studies report no sensations of illness in humans after physiologically relevant $PYY_{(3–36)}$ doses that inhibited 24-h food intake by 33% (15), indicating that illness is not a likely rationale for decreased appetite caused by $PYY_{(3–36)}$ infusion.

Although we are unable to make statistical comparisons on the day-to-day efficacy of $PYY_{(3–36)}$ to inhibit food intake, it generally inhibited food intake every day. The effect of $PYY_{(3–36)}$ on food intake may have diminished toward the end of the infusion, and further longer-term studies will be needed to test this. There is tremendous variability in the effects of any drug on primate ingestive behavior; seemingly trivial changes in their environment can cause monkeys to alter their normal behavior. Therefore, small deviations from a general effect should not be overinterpreted. However, the weight loss persisted even if the effect on food intake faded, as is seen with many weight loss pharmacotherapies.

Two weeks of twice-daily $PYY_{(3–36)}$ dosing had no effect on glucose clearance or insulin response to intravenous glucose during acute tests (results not shown). Only after spontaneous intake of a high-fat meal did total plasma PYY increase in proportion to calories. These results are consistent with the proposed release of PYY in response to ingested fat (26,27). If PYY release is mediated by an enteral, nutrient-dependent mechanism (26,28,29), it is possible that the effects of $PYY_{(3–36)}$ infusion on appetite and body weight may be greater in animals consuming a high-fat diet.

The results presented here demonstrate that $PYY_{(3–36)}$ transiently suppresses food intake and reduces body weight in monkeys, suggesting that targeting the PYY/Y2 system in humans may yield similar positive results. Because no physiological $PYY_{(3–36)}$ doses significantly reduced total meal consumption, it is unlikely that $PYY_{(3–36)}$ is a primary regulator of feeding behavior. Rather, our data suggest that postprandial $PYY_{(3–36)}$ is part of an anthology of neurohormonal signals that regulate appetite. Pharmacological activation of the NPY/Y2 receptor will probably be more efficacious in individuals with deficiencies in endogenous Y2 ligands [improper $PYY_{(1–36)}$ release or impaired enzymatic cleavage to $PYY_{(3–36)}$] or obese humans with lower PYY tone (14). This type of therapy would be in accordance with the precedent set by the high efficacy of leptin replacement therapy for weight loss in leptin-deficient humans (30,31). Nevertheless, our data do not completely dismiss $PYY_{(3–36)}$-based therapies. NPY-Y2 receptor activation may enhance the efficacy of other weight-loss treatments in overweight/obese patients with normally functioning PYY/Y2 systems and in patients who consume high-fat foods.

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