Effect of subcutaneous injections of PYY$_{1-36}$ and PYY$_{3-36}$ on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males

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Sloth B, Davidsen L, Holst JJ, Flint A, Astrup A. Effect of subcutaneous injections of PYY$_{1-36}$ and PYY$_{3-36}$ on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males. Am J Physiol Endocrinol Metab 293: E604–E609, 2007. First published June 12, 2007; doi:10.1152/ajpendo.00153.2007—Intravenous (iv) PYY$_{3-36}$ infusions have been reported to reduce energy intake (EI) in humans, whereas few studies exist on effects of PYY$_{1-36}$. The aim of the present study was to examine effects of subcutaneous (sc) injections of PYY$_{1-36}$ and PYY$_{3-36}$ on appetite, ad libitum EI, plasma concentrations of PYY and free fatty acids (FFA) in obese males. Twenty-four males (BMI 27–40 kg/m²) were randomly assigned to two groups receiving sc injections of either PYY$_{1-36}$ or PYY$_{3-36}$ in a blinded, placebo-controlled, dose-escalating, cross-over study. Subjects were studied 5 days in succession, with escalating doses of PYY$_{1-36}$ (saline, 50, 100, and 200 pmol PYY$_{1-36}$/kg lean body mass [LM]), or PYY$_{3-36}$ (saline, 25, 50, 75, and 100 pmol PYY$_{3-36}$/kg LM), respectively. PYY injections resulted in dose-dependent increases in plasma PYY levels but no effect on EI in either the PYY$_{1-36}$ or the PYY$_{3-36}$ group. However, increasing doses of PYY$_{3-36}$, but not PYY$_{1-36}$, resulted in increased ratings of satiety and decreased ratings of hunger, thirst, and prospective food consumption. Although not dose dependent, significant elevation of plasma FFA was seen after injection of PYY$_{1-36}$, but not PYY$_{3-36}$. Although sc administration of PYY was well tolerated, it remains to be determined whether high-dose PYY$_{3-36}$ is sufficient in reducing EI in long-term trials, and if so, whether the reduction in EI occurs without nausea. PYY$_{1-36}$ is unlikely to be important in regulating energy intake. The PYY$_{3-36}$ administrations caused a non-dose-dependent mobilization of FFA, likely through a direct effect.

peptide YY; lipolysis; catecholamine; hunger; satiety

IT IS WELL KNOWN that we are in the midst of an obesity epidemic with the associated pathological features resulting in serious consequences for human health and health-related economics. The search for potent antiobesity drug targets is therefore important and has in recent years focused on peptides altering human appetite. Peptide YY (PYY) is an endogenous 36-amino acid peptide found in endocrine cells of the gastrointestinal mucosa and in the circulation (1). It is released postprandially as PYY$_{1-36}$ and cleaved by dipeptidyl peptidase-IV to yield PYY$_{3-36}$ (9, 10). PYY$_{3-36}$ is believed to access neurons (3). PYY$_{1-36}$ has affinity for the Y1, Y2, and Y5 receptors, whereas PYY$_{3-36}$ binds more selectively to the Y2 receptor and with lower affinity to the Y5 receptor (17).

Human PYY$_{3-36}$ intravenous (iv) infusion studies have demonstrated dose-dependent (4, 12) decreases in energy intake (EI) of 23.36% at subsequent meals following 90 min of infusion at a rate of 0.8 pmol·kg$^{-1}·$min$^{-1}$ (2–4, 12). The quantity of this suppression of food intake and the fact that the effect has been shown to last for 12 h (2, 3), makes PYY$_{3-36}$ a promising antiobesity drug target. However, side effects in the form of nausea, vomiting, abdominal discomfort, fullness, and sweating have also been reported both with iv infusion (4, 16) as well as with nasal administration (8) of PYY$_{3-36}$, and at present it cannot be ruled out that part of the appetite suppressant effect of PYY$_{3-36}$ is due to nausea and discomfort.

Previous studies with iv infusion of PYY have not resulted in consistent increases of PYY plasma concentrations, and since the therapeutic window that results in reduced energy intake but no side effects seems to be narrow, PYY therapy will require methods of administration that produce a stable plasma level within this therapeutic range.

We (16) previously demonstrated increased thermogenesis, lipolysis, and increased postprandial insulin and glucose responses following PYY$_{3-36}$ iv infusions. The mechanisms behind these findings are still unclear but could involve increased sympathetic activity, a hypothesis that could be confirmed or rejected with catecholamine measurement following PYY$_{3-36}$ administration.

The aim of the present study was to examine the effect of subcutaneous (sc) injections of PYY$_{1-36}$ and PYY$_{3-36}$ on appetite, ad libitum EI, plasma concentrations of PYY, free fatty acids (FFA), and catecholamines in obese males.

METHODS

Subjects. Twenty-four healthy, weight-stable, obese Caucasian men were randomly assigned to either PYY$_{1-36}$ or PYY$_{3-36}$ administration. Their mean (±SD) ages were 38 ± 6 and 41 ± 6 yr, BMIs were 29.6 ± 2.5 and 32.2 ± 3.5 kg/m², and percentages of body fat mass were 32 ± 3 and 34 ± 4% in the PYY$_{1-36}$ and PYY$_{3-36}$ groups, respectively. All subjects signed a consent declaration after having received written and oral information about the study Protocol. The study was approved by the Municipal Ethics Committee of Copenhagen, Frederiksberg, and Zealand (registration no. 01-091/04) and was carried out in accordance with the Helsinki II declaration. The study is registered at http://www.clinicaltrials.gov.

Protocol. The study was performed in a dose-escalating, blinded, placebo-controlled manner with each subject studied for 5 days in
succession. The subjects were told to refrain from alcohol and strenuous exercise 24 h before and during the whole study period and were fasted from 2000 (including no water from midnight) the night before each test day. On test days, subjects arrived at the Department at 0730 having used nonstrenuous transportation. A venflon catheter was inserted into an antecubital arm vein for collection of blood samples. Subsequently, subjects relaxed for 10 min before baseline measurements and blood sampling was performed. At 0830, the peptides were administered by sc injection. Subjects rested in a supine position with the head slightly elevated for at least 10 min before each measurement or blood sampling was performed.

One-hundred-millimeter visual analog scales (VAS) with words anchored at each end, expressing the most positive and the most negative rating, were used to assess satiety, hunger, fullness, prospective food consumption, well-being, nausea, and thirst (7).

At 1200, an ad libitum lunch consisting of a pasta salad representing the average Danish diet (6), with 34 E% fat, 50 E% carbohydrate, and 16 E% protein, was served. At 1300, subjects left the laboratory and subsequently completed a food diary with listing of weights of all consumed food items until 2000, when time fasting began again. The food diaries were analyzed by a dietician with the aid of Dankost 3000 (Dansk Catering Center, Herlev, Denmark (14)). Water was served to subjects at specific times during the test day, and the amount drunk was repeated on subsequent test days.

**Injections.** The PYY1-36 and PYY3-36 peptides were purchased from Bachem (Weil am Rhein, Germany) and Polypeptide (Wolfenbüttel, Germany). All peptides were dissolved in 9 g/l sodium chloride and 2.3 g/l acetic acid and filled into nonsiliconized 2R glass vials (Apodan 712026; ApodanNordic, Copenhagen, Denmark) with non-telfon-coated bromobutyl rubber stoppers (Apodan 712638) after sterile filtration under aseptic conditions at the Central Pharmacy, Herlev, Denmark. Vial content was tested for sterility and bacterial endotoxins (Ph.Eur. 2.6.14, Method C, Turbidimetric kinetic method), and content was verified by sequence, mass, and amino acid analysis.

Vials were kept at −20°C. The doses of PYY were given as picomoles per kilogram lean body mass (LBM), which was calculated on the basis of body composition determined by bioelectrical impedance (Animeter; Unitech, Hobro, Denmark) on the first test day, using Resting ECG Electrodes Product (code 3000; Lectec, Minnetonka, MN) (13). The site of sc injection was the same on each study day, namely 10 cm laterally from the umbilicus on the right side of the abdomen. The injection was given with a 1 ml of Myojector (Terumo, MN) (13). The site of sc injection was the same on each study day, namely 10 cm laterally from the umbilicus on the right side of the abdomen. The injection was given with a 1 ml of Myojector (Terumo, Leuven, Belgium) at a 90° angle and no more than 1 cm into the abdomen to ensure that the injection remained subcutaneous. Two subjects in the PYY1-36 group were dosed with 0, 12.5, 25, 50, and 100 pmol/kg LBM in a pilot study; thereafter 10 subjects were dosed with 0, 50, 100, 150, and 200 pmol/kg LBM. The outline was similar in the PYY3-36 group, where initially two subjects were given 0, 12.5, 25, 50, and 100 pmol/kg LMB and thereafter 10 subjects were given 0, 25, 50, 75, and 100 pmol/kg LBM. Saline (9 g/l sodium chloride + 2.3 g/l acetic acid) was used for placebo injections at the same volume as the lowest PYY dose.

**Blood samples and analysis.** Blood was drawn without stasis through an indwelling antecubital cannula into iced chilled syringes (Vacutte; Greiner Labortechnik, Kremsmünster, Austria). Syringes for PYY and FFA contained EDTA, and syringes for catecholamines contained EDTA and gluthathione. All samples were centrifuged within 30 min for 10 min at 2,800 g at 4°C and stored at −20 or −80°C (catecholamine samples) until analysis.

Plasma concentrations of FFA were determined by use of an enzymatic colorimetric method using a Cobas Mira Plus spectrophotometer (Roche Diagnostic Systems, Basel, Switzerland). Plasma FFA concentrations were determined using a NEFA-C test kit (ACSCOD method; Wako chemicals, Neuss, Germany) with intra- and interassay CVs of <4.5 and <4.2%, respectively.

Radioimmunoassay of PYY in plasma was performed using antisem code no. 8412–2III (5). It reacts equally with PYY1-36 and PYY3-36. Synthetic human PYY1-36 or PYY3-36 (Peninsula Laboratories, UK) were used for standards as appropriate. 125I-labeled PYY3-36 (code no. IM259) was from Amersham Biosciences. Assay buffer was 0.05 mol/l sodium phosphate, pH 7.5, containing in addition 400 KIE/ml Trasylol-aprotinin, 0.1 mol/l sodium chloride, 10 mmol/l EDTA, 0.6 mmol/l merthiolate. One hundred fifty microliters of unknown plasma samples plus 150 µl of assay buffer or 150 µl of charcoal-treated plasma plus 150 µl of standards were preincubated with antiserum, 100 µl, diluted 1:20,000 (final concentration), for 48 h at 4°C. Then, 100 µl of tracer (5 fmol, specific activity 70 MBq/nmol) was added and the mixture incubated for 24 h before and free peptide moieties were separated by plasma-coated charcoal (11). Detection limit of the assay was <2.5 pmol/l. Plasma concentrations of plasma PYY added to plasma in concentrations between 5 and 50 pmol/l deviated less than 15% from expected values. Intra-assay CV was <5%. The antiserum showed no cross reaction with human NPY or human pancreatic peptide (PP) in concentrations up to 500 pmol/l.

Plasma concentrations of epinephrine and norepinephrine were determined in the PYY3-36 group following 50 pmol/kg PYY3-36 and placebo administration. These samples were measured in duplicate by enzyme immunoassay using a commercially available assay kit (2 CAT EIA; Labor Diagnostika Nord & KG, Nordhorn, Germany) relying on the conversion of extracted catecholamines to acetylated derivatives. Sensitivities were 11 and 44 pg/ml and intra-assay CV of 7 and 10% at 2.5 and 92 ng/ml, respectively. For both assays, quality controls fell within accepted limits. Due to technical reasons, samples from only six subjects were analyzed for norepinephrine, whereas epinephrine analysis was done for all 12 subjects.

**Statistical analysis.** Differences between test days in EI were analyzed using ANOVA with dose as the main factor and subject as the random factor. The effects of saline, PYY1-36, and PYY3-36 on plasma PYY concentration, plasma FFA concentration, and VAS ratings were compared within the two different subject groups using ANCOVA with repeated measurements with subject, dose, time, and dose × time as main factors and baseline values as covariate, taking autocorrelation into account. The dose effect was split into a linear (d) and a nonlinear (dose) part, for both the main effects and the interaction with time, in order to gain strength of the test. Only one test showed significance in the nonlinear part (plasma FFA in the PYY3-36 group). All other tests proved the nonlinear part to be nonsignificant. When this was the case, the model was reduced to a model dealing only with the linear part of the dose effect (d). When factors were nonsignificant, the models were reduced successively.

The model control was performed with an unstructured covariance matrix. In general, this model control demonstrated the desired covariance structure for the data before the ad libitum meal (time 0–180 min). However, the 210- and 240-min values deviated from the desired pattern. For this reason, the tests were performed first with all data (time 0–240 min) and then with premeal values (time 0–180 min). In general, the conclusions for these two tests were not significantly different; therefore, only P values from analysis that includes all data (time 0–240 min) are presented.

The statistical tests were performed with the aid of SAS version 8 (SAS Institute, Cary, NC). Results are presented as means ± SE.

**RESULTS**

**Plasma PYY concentrations.** There was a significant dose-dependent increase in plasma PYY concentrations in both the PYY1-36 and the PYY3-36 groups (Fig. 1). Plasma PYY levels peaked 30 min after the sc injection of PYY1-36 and ranged from 34 ± 4 pmol/l with the lowest dose to 90 ± 6 pmol/l with the highest dose. Likewise, plasma PYY peak levels occurred 30–45 min after the sc injection of PYY3-36 and ranged from
were not significantly reduced with either the PYY$_{1-36}$ or the PYY$_{3-36}$ administrations (Fig. 2).

**Appetite ratings.** There were no significant differences in the ratings of satiety, hunger, fullness, and prospective food consumption when the 5 test days were compared in the PYY$_{1-36}$ group (data not shown). A significant difference between the 5 test days in thirst was found in the PYY$_{1-36}$ group, but there was no clear dose-related pattern (data not shown).

In the PYY$_{3-36}$ group ratings of satiety, hunger, and prospective food consumption were significantly different when the 5 test days were compared (Fig. 3), whereas ratings of fullness were not different ($P_d = 0.19$, data not shown). There was a linear, dose-dependent effect with an estimated increase in ratings of satiety of 2.0 ± 0.1 mm with every increase in dose. This resulted in a total difference of 8.0 ± 0.1 mm between the saline injection and the highest dose of PYY$_{3-36}$ (100 pmol/kg LBM). Ratings of hunger were associated with an inverse dose-response effect with an estimated decrease of 2.6 ± 0.1 mm with every increase in dose, which resulted in a total difference of 10.4 ± 0.1 mm between saline injection and the highest dose of PYY$_{3-36}$. Likewise, ratings of prospective food consumption decreased 2.1 ± 0.1 mm with every increase in dose and this resulted in a total difference of 8.4 ± 0.1 mm between saline and the highest dose of PYY$_{3-36}$.

Finally, an inverse dose-response effect appeared in ratings of thirst with an estimated decrease of 2.5 ± 0.1 mm with every increase in dose, which led to a total decrease in thirst of 10.1 ± 0.1 mm between saline injection and the highest dose of PYY$_{3-36}$ ($P_d = 0.003$; data not shown).

**Fig. 2.** Mean ± SE ad libitum energy intake (EI) from a test meal served 180 min after sc injection of PYY$_{1-36}$ (A) and PYY$_{3-36}$ (B). Ad libitum meal was served at time 180 min. FFM, fat-free mass. Data were analyzed with ANCOVA with repeated measurements with linear effect of dose (d). $P$ values arise from all data (time 0–240). PYY$_{1-36}$: $P_d$ time $= 0.0001$; PYY$_{3-36}$: $P_d$ time $< 0.0001$.

**Fig. 1.** Mean ± SE plasma peptide YY (PYY) concentrations from baseline to 240 min after sc injection of saline, PYY$_{1-36}$ (A), and PYY$_{3-36}$ (B). Ad libitum meal was served at time 180 min. FFM, fat-free mass. Data were analyzed with ANCOVA with repeated measurements with linear effect of dose (d). $P$ values arise from all data (time 0–240). PYY$_{1-36}$: $P_d$ time $= 0.0001$; PYY$_{3-36}$: $P_d$ time $= 0.0001$.
There was no difference in plasma FFA concentration when the 5 test days were compared in the PYY1-36 group (Pd/H11005 0.31; data not shown). However, a significant difference in plasma FFA concentration was found between the 5 test days in the PYY3-36 group. This difference however, was not dose dependent but could be explained by the difference between doses of 25 and 50 pmol/kg LBM. Administration of 25 pmol/kg LBM resulted in the lowest plasma FFA concentration, even below that of the placebo day. This was increased to the highest level detected among the four doses when 50 pmol/kg LBM was administered. Subsequently, doses of 75 and 100 pmol/kg LBM resulted in plasma FFA concentrations below those reported after administration of 50 pmol/kg LBM but above those reported on the placebo day and after 25 pmol/kg LBM (Fig. 4). Catecholamine concentration did not increase following 50 pmol/kg LBM PYY3-36 administration (data not shown).

**DISCUSSION**

Our study demonstrates that sc administration of PYY produces predictable dose-dependent increases in circulating PYY with only few and mild side effects.

In contrast to previous findings (2–4, 12, 15), we found no effect on ad libitum EI following either of the peptides, regardless of dose. We cannot exclude the possibility that the dose-escalating design of our study may be partly responsible for this. In general, subjects tend to feel more comfortable with a study protocol after experiencing the test day more than once, and this may increase their EI and thus blunt an inhibitory effect of the peptides. Development of tolerance to the peptides can also not be ruled out as a reason for lack of effect on EI. VAS ratings of appetite demonstrated that increasing doses of PYY3-36, but not PYY1-36, resulted in enhanced satiety and less hunger, thirst, and perceived ability to consume food. These findings on PYY3-36 are in accord with previous studies (2–4, 12) also demonstrating decreased hunger and fullness and increased satiety following PYY3-36 iv infusions. In general, large differences are seen among the previous iv infusion studies (2–4, 12, 15, 16) with regard to plasma PYY concentrations. This may to some extent be due to differences in the assays

**FFA and catecholamine concentrations.** There was no difference in plasma FFA concentration when the 5 test days were compared in the PYY1-36 group (Pd = 0.31; data not shown). However, a significant difference in plasma FFA concentration was found between the 5 test days in the PYY3-36 group. This difference however, was not dose dependent but could be explained by the difference between doses of 25 and 50 pmol/kg LBM. Administration of 25 pmol/kg LBM resulted in the lowest plasma FFA concentration, even below that of the placebo day. This was
used for PYY analysis. However, both our infusion study (16) and the study by Degen et al. (4) resulted in side effects after PYY3-36 infusion, primarily in the form of nausea and vomiting, whereas studies with lower plasma concentrations have not reported these side effects (2, 3, 12, 15). Previous iv infusion studies with different doses of PYY3-36 suggest that the plasma PYY concentration must reach a certain level to result in a reduced food intake (4, 12). This, taken together with the studies demonstrating side effects (4, 16) with higher plasma PYY concentrations, suggests that the therapeutic window for PYY3-36 treatment is narrow and nausea might well be part of the EI-lowering mechanism.

In the present study, only a few and milder side effects were observed even though plasma levels of PYY reached 90 and 112 pmol/l following highest dose PYY1-36 and PYY3-36 administration, respectively. The fewer side effects, compared with what has been reported in previous iv infusion studies (4, 16), could be speculated to be due to the dose escalation design of our study, in which subjects probably tend to develop a tolerance to the peptides, possibly affecting both side effects and EI. Also, the slower increase in the plasma concentration of PYY after sc compared with iv infusion could play a role. It is of interest that the sc mode of administration caused an elevation of PYY concentrations for more than 4 h for the higher doses, which could be speculated to result in a long-lasting appetite-suppressive effect, which would probably be desirable in the therapeutic setting.

In our previous iv infusions studies, we found significant 182 and 95% increases in FFA concentrations following low- and high-dose PYY3-36 administration, respectively (16). In the present study, the increasing effect on FFA of PYY3-36 was not dose dependent and might therefore suggest that there is a threshold plasma concentration for lipolytic activation. On the basis of the data in our previous infusions studies, we speculated that the lipolytic effect of PYY3-36 could be due to an activation of the sympathetic nervous system, but no elevation of catecholamine concentrations was found following the dose that produced the greatest lipolytic effect in the present study. This finding suggests that the earlier reported lipolytic action of PYY3-36 is a direct effect, and the mechanisms behind this effect are unknown.

In summary, sc administration of PYY1-36 and PYY3-36 at four different doses produced no difference in ad libitum EI, but the dose-escalating study design could be a confounding factor. Subcutaneous administration of PYY3-36, but not PYY1-36, was able to dose-dependently induce lower subjective hunger and thirst ratings and higher satiety ratings. The PYY1-36 administrations resulted in a non-dose-dependent increase of FFA, likely through a direct effect, which calls for future studies. In view of the small number of side effects and the reproducibly prolonged plasma PYY elevation, sc administration of PYY seems to be a suitable route of administration for future long-term trials examining effects of PYY on body weight. However, the therapeutic window for PYY3-36 treatment is narrow, and it remains to be determined whether high-dose PYY3-36 is sufficient in reducing EI in long-term trials, and if so, whether the reduction in EI occurs without nausea. PYY1-36 is unlikely to be important in regulating energy intake.

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DISCLOSURES

None of the authors have any conflict of interest. A. Astrup was in 2004–2005 a consultant for Aditech Pharma AB, which has patents pending for PYY. J. J. Holst’s work is supported by the Novo Nordisk Foundation and by the Danish Medical Research Council.

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