Chronic PYY$_{3–36}$ treatment promotes fat oxidation and ameliorates insulin resistance in C57BL6 mice

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van den Hoek AM, Heijboer AC, Voshol PJ, Havekes LM, Romijn JA, Corssmit EP, Pijl H. Chronic PYY$_{3–36}$ treatment promotes fat oxidation and ameliorates insulin resistance in C57BL6 mice. Am J Physiol Endocrinol Metab 292: E238–E245, 2007. First published August 29, 2006; doi:10.1152/ajpendo.00239.2006.—PYY$_{3–36}$ is a gut-derived hormone acting on hypothalamic nuclei to inhibit food intake. We recently showed that PYY$_{3–36}$ acutely reinforces insulin action on glucose disposal in mice. We aimed to evaluate effects of PYY$_{3–36}$ on energy metabolism and the impact of chronic PYY$_{3–36}$ treatment on insulin sensitivity. Mice received a single injection of PYY$_{3–36}$ or were injected once daily for 7 days, and energy metabolism was subsequently measured in a metabolic cage. Furthermore, the effects of chronic PYY$_{3–36}$ administration (continuous and intermittent) on glucose turnover were determined during a hyperinsulinemic-euglycemic clamp. PYY$_{3–36}$ inhibited cumulative food intake for 30 min of refeeding after an overnight fast (0.29 ± 0.04 vs. 0.56 ± 0.12 g; P = 0.036) in an acute setting, but not after 7 days of daily dosing. Body weight, total energy expenditure, and physical activity were not affected by PYY$_{3–36}$. However, it significantly decreased the respiratory quotient. Both continuous and intermittent PYY$_{3–36}$ treatment significantly enhanced insulin-mediated whole body glucose disposal compared with vehicle treatment (81.2 ± 6.2 vs. 77.1 ± 5.2 vs. 63.4 ± 5.5 μmol·min$^{-1}·$kg$^{-1}$, respectively). In particular, PYY$_{3–36}$ treatment increased glucose uptake in adipose tissue, whereas its impact on glucose disposal in muscle did not attain statistical significance. PYY$_{3–36}$ treatment shifts the balance of fuel use in favor of fatty acids and enhances insulin sensitivity in mice, where it particularly promotes insulin-mediated glucose disposal. Notably, these metabolic effects of PYY$_{3–36}$ remain unabated after chronic administration, in contrast to its anorexic effects.

diabetes; brain; metabolism; gut hormone

THE METABOLIC SYNDROME comprises a cluster of anomalies that increase the risk of cardiovascular disease and type 2 diabetes mellitus: hyperglycemia, abdominal obesity, hypertriglyceridemia, hypertension, and low levels of HDL cholesterol (23, 34, 37). Insulin resistance may underlie the majority of these pathologies (11), and therapies that effectively reinforce insulin action may therefore ameliorate the risk profile of metabolic syndrome patients (29, 40).

Diet-induced obese, insulin-resistant C57BL6 mice have increased levels of neuropeptide Y (NPY) and decreased levels of proopiomelanocortin (POMC) in hypothalamic nuclei (16, 17, 25). These neuropeptides have metabolic effects, which suggest that these features of hypothalamic neural circuits may be involved in the pathogenesis of the metabolic syndrome. First of all, NPY and POMC are critical players in the control of energy balance, where NPY stimulates feeding, lowers body temperature, inhibits oxygen consumption, and elevates the respiratory quotient (RQ) (8, 19, 28, 45), whereas POMC exerts precisely opposite effects on these aspects of energy metabolism (15, 18, 30). Second, NPY induces insulin resistance of endogenous glucose production, and POMC neural circuits facilitate insulin action. (26, 32, 47, 49). Therefore, antagonists of NPY and/or agonists of POMC signaling may be useful tools in the clinical management of the metabolic syndrome.

Peptide YY$_{3–36}$ (PYY$_{3–36}$) is released in response to food intake by L cells in the distal gastrointestinal tract. It acts via Y2 receptors on NPY neurons in the arcuate nucleus to inhibit feeding. In that context, we wondered whether the effects of PYY require specific feeding conditions. To mimic the physiological changes in PYY$_{3–36}$ that occur in response to a meal, a low dose of PYY$_{3–36}$ was used. Because the physiology of PYY$_{3–36}$ entails intermittent release in response to food intake, we also examined whether continuous and intermittent administration of PYY$_{3–36}$ impacts glucose metabolism differentially.

METHODS

Animals

Male C57BL6 mice were housed in a temperature-controlled room on a 12:12-h light-dark cycle with free access to water and were fed either a standard mouse chow diet (metabolic cage experiments) or a standard mouse chow diet (metabolic cage experiments) or a standard mouse chow diet (metabolic cage experiments) or a standard mouse chow diet (metabolic cage experiments).
high-fat diet (43% energy fat derived from bovine lard; Hopefarms, Woorden, The Netherlands) for 16 wk to induce insulin resistance (hyperinsulinemic clamp experiments). All animal experiments were performed in accordance with the regulations of Dutch law on animal welfare, and the institutional ethics committee for animal procedures approved the protocol.

Experiments to Determine Effects of PYY3–36 on Energy Metabolism

Metabolic cages. Oxygen consumption, CO2 production, energy expenditure, heat production, food intake, and activity were measured using a Comprehensive Laboratory Animal Monitoring System (Columbus Instruments, Columbus, OH). An eight-chamber open-circuit system was used. The mice were housed individually in plexiglass cages through which 0.6 liters of air was passed per min. Detectors measured O2 and CO2 sequentially across each chamber for 45 s. RQ was calculated as the volume of CO2 produced (VCO2) divided by the volume of O2 consumed (VO2). Analyzers were calibrated before each use with primary gas standards of high purity. Mice were habituated to the metabolic cages during the months before the experiments by having them put in the cages for a few days >10 times. Before the start of each experiment, mice were again acclimated to the cages for 1 day. Metabolic parameters were measured after acute and chronic administration of PYY3–36.

Acute experiments. Sixteen male, ~7-mo-old mice fed regular chow received a subcutaneous injection of PYY3–36 (2.5 μg; Phoenix Pharmaceuticals, Belmont, CA) or vehicle at 9 AM. Measurements began immediately after the injection for a period of ≥90 min and were determined under different feeding circumstances. Mice were fasted the night preceding the injection and subsequently maintained fasted (experiment 1A) or refed after the injection with either free access to food (experiment 1B) or both groups (vehicle and PYY3–36 treated) pair-fed at the amount of food consumed by the mice treated with PYY3–36 (experiment 1C). In addition, measurements were made in mice that were normally fed on the night preceding the injection and subsequently fasted after the injection (experiment 1D).

Chronic experiments. Sixteen male ~11-mo-old mice, fed regular chow, received daily subcutaneous injections (50 μl at 9 AM) of saline or PYY3–36 (2.5 μg) for 7 days. During these days of treatment, access to food was free. Food was not available during the night preceding the last injection, whereas the animals were allowed to eat ad libitum after the injection (experiment 1E).

Experiments to Determine Effects of PYY3–36 on Insulin Action

Thirty male ~3-mo-old mice were fed a high-fat diet for 16 wk to induce insulin resistance. After 15 wk of a high-fat diet, mice that did not respond to the diet were left out. In the remaining mice, osmotic minipumps (Alzet minipump, model 2001; Charles River, Maastricht, The Netherlands) were placed subcutaneously in the back region under light isoflurane anesthesia. All mice received a saline or PYY3–36 (2.5 μg/day; Phoenix Pharmaceuticals) infusion via the osmotic minipump at a rate of 0.5 μl/h for 7 days. In addition, daily subcutaneous injections (50 μl at AM) of saline or PYY3–36 (2.5 μg) were given, where mice receiving continuous PYY3–36 treatment were additionally injected with saline, and mice receiving saline by minipump were assigned to receive either saline or PYY3–36 by injection. Thus mice were divided into three experimental groups: 1) receiving saline (n = 8), 2) receiving PYY3–36 continuously by minipump (n = 5), and 3) receiving PYY3–36 intermittently by daily subcutaneous injection (n = 7).

Mice fasted overnight with food were withdrawn at 5 PM the day prior to the study. The next morning at 9 AM, right after the last subcutaneous injection, hyperinsulinemic euglycemic clamps were performed as described earlier (46–48). In short, mice were anesthetized with 6.25 mg/kg acepromazine, (Sanofi sante animale, Libouine Cedex, France), 6.25 mg/kg midazolam (Roche, Mijdrecht, The Netherlands), and 312.5 μg/kg fentanyl (Janssen-Cilag, Tilburg, The Netherlands), and an infusion needle was placed in one of the tail veins. First, basal rates of glucose turnover were measured by giving a primed (0.7 μCi) continuous (1.2 μCi/h) infusion of [14C]glucose (Amersham, Little Chalfont, UK) for 80 min. Subsequently, insulin was administered in a primed (4.1 μU) continuous (6.8 μU/h) intravenous infusion for 2 to 3 h to attain steady-state circulating insulin levels of ~3.5 ng/ml. The [14C]glucose infusion (1.2 μCi/h) infusion was continued. A variable infusion of 12.5% d-glucose was also started and adjusted to maintain euglycemia (measured at 10-min intervals via tail bleeding, <3 μL Freestyle, TheraSense; Disetronic Medical Systems, Vianen, The Netherlands). Blood samples (75 μl) were taken during the basal period (after 60 and 80 min) and during the clamp period (when glucose levels were stable and 20 and 40 min later) for determination of plasma glucose, free fatty acids (FFA), insulin, and PYY3–36 concentrations and [14C]glucose specific activities.

To assess insulin-mediated glucose uptake in individual tissues, 2-deoxy-d-[3H]glucose (2-[3H]DG; Amersham) was administered as a bolus (1 μCi) 40 min before the end of the clamp experiments. At the end of the clamp, mice were killed, and muscle and adipose tissue were isolated and frozen in liquid nitrogen for subsequent analysis.

Analytical Procedures

Plasma levels of glucose and FFA were determined using commercially available kits (Instruchemie, Delfzijl, The Netherlands, and Wako, Neuss, Germany). Plasma insulin and PYY3–36 concentrations were measured by using a mouse insulin ELISA and PYY3–36 RIA (sensitivity of 1 pg/ml for the PYY3–36 RIA; Mercodia, Uppsala, Sweden, and Phoenix Pharmaceuticals). Total plasma [14C]glucose was determined in 7.5 μl of plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water.

Tissue Analysis

For determination of tissue 2-DG uptake, the homogenate of muscle and adipose tissue was boiled and the supernatant subjected to an ion exchange column to separate 2-DG-6-P from 2-DG, as described previously (13, 38, 48).

Calculations

Turnover rates of glucose (μmol·min⁻¹·kg⁻¹) were calculated during the basal period and in steady-state clamp conditions as the rate of tracer infusion (dpm/min) divided by the plasma specific activity of [14C]glucose (dpm/μmol). The ratio was corrected for body weight. Endogenous glucose production (EGP) was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate.

Tissue-specific glucose uptake in muscle and adipose tissue were calculated from tissue 2-DG content, corrected for plasma specific activity, and expressed as μmol per gram of tissue.

Statistical Analysis

Since the number of mice in each group was rather small, we assumed that they were not normally distributed. Therefore, nonparametric tests were used. Differences between groups were determined by Kruskall-Wallis and Mann-Whitney tests for independent samples. A P value <0.05 was considered statistically significant. All values shown represent means ± SE.
Respiratory quotient (RQ) was measured in a 90-min test session after the injection of either vehicle or peptide YY3–36 (PYY3–36). Mice were maintained on a normal chow diet prior to the experiments.

- **A** - in the first acute study, mice were fasted the night preceding the injection and were subsequently maintained fasted after the injection.
- **B** - in the second acute study, mice were fasted the night preceding the injection and refed after injection. Cumulative food intake was measured for 30 min.
- **C** - in the third acute study, mice were fasted the night preceding the injection and refed after injection, where mice treated with vehicle were fed the same amount of food as the mice treated with PYY3–36.
- **D** - in the fourth acute study, mice were fed normally the night preceding the injection and subsequently fasted after injection.
- **E** - in the chronic study, mice received daily injections for 1 wk and were fasted the night preceding the last injection and subsequently refed after injection. Cumulative food intake was measured for 30 min.

Values represent means ± SEM of 6 mice/group. *P < 0.05 vs. vehicle.

Fig. 1. Respiratory quotient (RQ) was measured in a 90-min test session after the injection of either vehicle or peptide YY3–36 (PYY3–36). Mice were maintained on a normal chow diet prior to the experiments. **A**, **B**, **C**, **D**, **E**, **F**, **G**.
RESULTS

Experiments to Determine Effects of PYY3-36 on Energy Metabolism

Animals. A total of 16 animals were used in the energy metabolism experiments. Body weight did not differ before vs. after any of the experiments [acute experiments: fasted body weight before (mean body weight of experiments 1A–1C), 29.1 ± 0.8 vs. 28.1 ± 0.9 g; fed body weight before (experiment 1D), 31.8 ± 1.0 vs. 30.5 ± 0.7 g; body weight after (mean body weight of experiments 1A–1D), 29.6 ± 0.9 vs. 29.3 ± 1.3 g for vehicle- and PYY-treated animals, respectively] (chronic experiment: body weight before, 35.4 ± 1.4 vs. 36.0 ± 1.5 g; body weight after, 31.0 ± 1.3 vs. 32.9 ± 1.2 g for vehicle- and PYY-treated animals, respectively). Animals were maintained on a normal chow diet prior to (and during, if free access to food was allowed) the experiments. Animals that somehow managed to get access to the closed feeder during fasting conditions were excluded from the experiment.

Acute experiments. During each night preceding an injection, RQ, energy expenditure, heat production, and physical activity were similar in mice receiving PYY3-36 vs. control. PYY3-36 did not affect total energy expenditure, heat production, or physical activity in any study. In the first study (experiment 1A), all mice were fasted prior to injection and were maintained fasted after the injection. Due to the fasting, RQ levels were low and maintained low after the injection of PYY3-36/vehicle (Fig. 1A). In the second study (experiment 1B), all mice were fasted prior to injection and had free access to food after injection, and PYY3-36 significantly reduced 30-min cumulative food intake (0.29 ± 0.04 vs. 0.56 ± 0.12 g, P = 0.036, Fig. 1F). Moreover, RQ was low at baseline, and PYY3-36 significantly blunted the rise of RQ that occurred during (re)feeding in this experimental context (Fig. 1B). The reduction of RQ was not due to the difference in cumulative food intake between the groups, as evidenced by the third study (experiment 1C), where PYY3-36 induced a similar reduction of RQ in pair-fed conditions (Fig. 1C). In the fourth study (experiment 1D), mice had free access to food during the night prior to the injection and were subsequently fasted after the injection. Also under these feeding conditions, RQ was significantly reduced by PYY3-36 (Fig. 1D).

Chronic experiment. In this experiment (experiment 1E), mice received PYY3-36 once daily for 7 days and were fasted the night prior to the last (7th) injection, whereas they had free access to food after injection. Again, total energy expenditure, heat production, and physical activity were not affected by PYY3-36 as in the acute experiments. Interestingly, PYY3-36 did not inhibit food intake in this experimental context (Fig. 1G), suggesting that chronic administration blunts the initial anorectic effect of this peptide. However, PYY3-36 significantly curtailed the rise of RQ in response to refeeding (Fig. 1E), as it did in the acute experiments, which clearly indicates that chronic treatment does not blunt the effect of PYY3-36 on fuel oxidation.

Experiments to Determine Effects of PYY3-36 on Insulin Action

Animals. A different set of animals was used for the insulin action experiments. Body weight did not differ between animals that received continuous PYY3-36 administration, intermittent PYY3-36 administration, or vehicle [before treatment: 35.5 ± 2.1 vs. 32.1 ± 1.6 vs. 33.9 ± 0.9 g (P = 0.43); body weight after 7 days of PYY3-36/saline administration: 29.5 ± 1.8 vs. 26.9 ± 1.3 vs. 28.3 ± 0.5 g (P = 0.39) for the continuous PYY3-36 group, the intermittent PYY3-36 group, and the control group, respectively]. Animals were maintained on a high-fat diet for 16 wk prior to the hyperinsulinemic euglycemic clamp experiments to induce insulin resistance. Overnight food intake was measured on day 2 and day 5 of PYY3-36/saline administration and was similar in all groups (day 2: 2.14 ± 0.98 vs. 2.48 ± 0.56 vs. 2.32 ± 0.33 g, P = 0.52; day 5: 2.53 ± 0.69 vs. 2.92 ± 0.39 vs. 2.75 ± 0.43 g, P = 0.42, in continuous PYY3-36*, intermittent PYY3-36*, and vehicle-treated animals, respectively).

Plasma parameters. Plasma glucose, FFA, and insulin concentrations in basal and hyperinsulinemic conditions are shown in Table 1. Plasma glucose and insulin concentrations did not differ between animals treated with vehicle vs. those treated with either continuous PYY3-36 or intermittent PYY3-36 under basal or steady-state clamp conditions. Also, continuous and intermittent PYY3-36 administration had a similar impact on all of these parameters except for the plasma glucose levels under basal conditions, which were slightly but significantly higher in the group that received continuous PYY3-36 administration (P = 0.048).

 Plasma FFA concentrations were not significantly different between groups in basal conditions (P = 0.077) but were significantly decreased in steady-state clamp conditions in the mice that received PYY3-36, intermittently compared with vehicle-treated mice (P = 0.017).

 Plasma PYY3-36 concentrations in basal and hyperinsulinemic conditions were below the detection level in all groups (<1 pg/µl) except for the basal condition of the mice that received PYY3-36 intermittently (3.7 ± 0.8 pg/µl).

Glucose turnover. In basal conditions, glucose disposal/endogenous glucose production was similar in all groups (55.8 ± 5.8 vs. 49.3 ± 12.6 vs. 50.4 ± 10.4 µmol·min⁻¹·kg⁻¹ in continuous PYY3-36*, intermittent PYY3-36*, and vehicle-treated animals, respectively, P = 0.44). The rate of glucose

Table 1. Plasma parameters

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<th>Hyperinsulinemic</th>
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<td>Vehicle</td>
<td>Continuous PYY</td>
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<td>Glucose, mmol/l</td>
<td>7.7 ± 0.5</td>
<td>9.3 ± 0.4*</td>
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<tr>
<td>FFA, mmol/l</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.1</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
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Data are means ± SE. PYY, peptide YY; FFA, free fatty acids. Plasma parameters under basal or hyperinsulinemic conditions in overnight-fasted mice that received vehicle (n = 8) or PYY3-36 continuously (n = 5) or intermittently (n = 7) for 7 days. *P < 0.05 vs. vehicle; †P < 0.05 vs. intermittent PYY3-36.
infusion necessary to maintain euglycemia during insulin infusion was significantly higher in both of the PYY3–36-treated groups compared with the vehicle-treated group [55 ± 4 vs. 54 ± 4 vs. 33 ± 4 μmol·min⁻¹·kg⁻¹ in continuous PYY3–36⁻ (P = 0.011), intermittent PYY3–36⁻ (P = 0.001), and vehicle-treated animals, respectively; Fig. 2A], indicating that chronic PYY3–36 administration enhances whole body insulin sensitivity.

Hyperinsulinemic glucose disposal rate was significantly higher in both the PYY3–36-treated groups compared with the vehicle-treated group [81.2 ± 6.2 vs. 77.1 ± 5.2 vs. 63.4 ± 5.5 μmol·min⁻¹·kg⁻¹ in continuous PYY3–36⁻ (P = 0.040), intermittent PYY3–36⁻ (P = 0.037), and vehicle-treated animals, respectively; Fig. 2B].

The EGP under hyperinsulinemic conditions was similar in all groups (26.5 ± 6.3 vs. 23.5 ± 4.3 vs. 30.1 ± 5.5 μmol·min⁻¹·kg⁻¹ in continuous PYY3–36⁻, intermittent PYY3–36⁻, and vehicle-treated animals, respectively, P = 0.70; Fig. 2C) and was suppressed by insulin to the same extent in all groups (by 52 ± 11 vs. 55 ± 5 vs. 40 ± 9% inhibition from basal in continuous PYY3–36⁻, intermittent PYY3–36⁻, and vehicle-treated animals, respectively, P = 0.46).

Tissue-specific glucose uptake. Insulin-mediated 2-DG uptake was measured in muscle and adipose tissue. In muscle, 2-DG was similar in all groups [4.0 ± 1.1 vs. 3.6 ± 0.5 vs. 2.6 ± 0.5 μmol/g tissue in continuous PYY3–36⁻, intermittent PYY3–36⁻, and vehicle-treated animals, respectively, (P = 0.24), Fig. 3A]. In adipose tissue, 2-DG uptake was significantly increased in both PYY3–36-treated groups compared with the vehicle-treated group [0.6 ± 0.1 vs. 0.5 ± 0.1 vs. 0.3 ± 0.1 μmol/g tissue in continuous PYY3–36⁻ (P = 0.042), intermittent PYY3–36⁻ (P = 0.032), and vehicle-treated animals, respectively, Fig. 3B].

**DISCUSSION**

Here, we show that acute and chronic PYY3–36 administration reduces RQ in C57BL6 mice, implying a shift in substrate utilization from carbohydrate toward fat oxidation in the fed condition. This appears to occur through a mechanism that is independent of the capacity of PYY3–36 to reduce food intake. Furthermore, PYY3–36 improves whole body insulin sensitivity of glucose metabolism. In particular, PYY3–36 treatment enhances the ability of insulin to promote glucose disposal via mechanistic routes that are independent of food intake or body weight. Continuous and intermittent administration of PYY3–36 reinforces insulin action to a similar extent. Interestingly, the clear anorectic effect of PYY3–36 no longer occurs after chronic administration, whereas its metabolic effects remain unaffected after 7 days of treatment.

**Effects of PYY3–36 on Energy Metabolism**

PYY3–36 consistently reduced RQ, and this metabolic effect was unabated after 1 wk of daily dosing. Because RQ reflects the relative amounts of fat and carbohydrate oxidized, whereas low RQ indicates predominance of fat oxidation, our data suggest that PYY3–36 shifts the balance of fuel use in favor of fat. Notably, the effect was measurable for ~90 min after injection (Fig. 1, B–E), which corroborates previous reports (43) showing persistent functionality of exogenous PYY3–36 despite a very brief plasma half-life. In contrast, PYY3–36 did
not affect total energy expenditure, heat production, or physical activity in any of the experiments reported here. A very recent paper similarly reports that chronic PYY3–36 treatment reduces RQ without affecting energy expenditure in C57BL/6J mice maintained on a high-fat diet (1). The authors suggested that their observations may be due to the concomitant anorexic effect of PYY3–36. However, we show here that this is not the case, as evidenced by the fact that pair feeding did not affect the impact of PYY3–36 on RQ. Alternatively, PYY3–36 may have reduced the rate of gastric emptying in the present experiments and thereby delayed the postprandial rise of RQ. Whatever the mechanism, 2.5 µg of PYY3–36 daily shifts the postprandial substrate oxidation balance but leaves total energy expenditure unaffected in C57BL6 mice.

PYY3–36 clearly inhibited food intake during refeeding in response to an overnight fast by more than 50% (Fig. 1F). Notably, PYY3–36 diminished food intake during refeeding after an overnight fast, but it did not affect 24-h food intake or body weight. This is in keeping with previous reports [4, 5 (supporting figures), 33] showing that this dose of PYY3–36 (2.5 µg·mouse−1·day−1, ~10 µg/100 g, or ~100 µg·kg−1·day−1) inhibits food intake only after overnight fasting and not during normal feeding conditions in mice. A much higher dose has been shown to inhibit 24-h food intake in normal feeding conditions and decrease body weight during a 7-day treatment (1, 33), implying that the effects of PYY3–36 on (24-h) food intake and body weight are dose dependent. Notably, the anorexic effect of PYY3–36 in the refeed condition was no longer measurable after 1 wk of daily PYY3–36 injections (Fig. 1G). These findings corroborate previous reports documenting disappearance of the acute anorexic effect of PYY3–36 after repeated administration (1, 4). PYY3–36 impacts food intake via Y2 receptor-mediated modulation of peptidergic neuronal activity in the arcuate nucleus, where inhibition of NPY gene transcription and peptide release may be of primary importance in this context (3, 4). Therefore, our observations are also in keeping with reports documenting transitory effects of NPY antagonistic drugs on feeding (7, 9, 21).

In concert, the data presented so far suggest that the anorexic effect of PYY3–36 wanes after a couple of daily doses, whereas its impact on fuel oxidation persists.

Effects of Subchronic Administration of PYY3–36 on Insulin Action

Our data also show that chronic PYY3–36 treatment enhances the ability of insulin to promote glucose disposal, which corroborates our previous findings that unveil similar acute effects of PYY3–36 administration on insulin action (46). In particular, PYY3–36 facilitates glucose uptake in adipose tissue. Disposal in muscle, which may be of more physiological relevance, tended to also be enhanced, but this effect did not quite reach statistical significance.

Although PYY3–36 enhanced insulin-induced glucose disposal, it did not significantly affect the ability of insulin to inhibit endogenous glucose production. However, the experimental group size and intersubject variation of EGP may have limited the statistical power necessary to significantly detect differences in insulin’s capacity to suppress EGP. Thus we cannot exclude the possibility that PYY3–36 also impacts hepatic glucose metabolism. Alternatively, PYY3–36 exerts differential, tissue-specific effects on insulin action.

During insulin infusion, average circulating insulin levels appeared somewhat higher in the PYY3–36-treated animals, but the difference with levels in control animals was far from statistically significant (P = 0.517). Nevertheless, high insulin levels could obviously explain increased glucose uptake in PYY3–36-treated animals. However, even if corrected for circulating insulin concentrations, glucose disposal remained significantly enhanced in PYY3–36-treated groups (data not shown). It is also important to emphasize that the metabolic effects of PYY3–36 treatment occur without measurable changes in food intake or body weight. Thus the data support the emerging concept of neural circuits controlling fuel flux through mechanisms that are independent of their impact on energy balance. Moreover, our data indicate that the metabolic effects of PYY3–36 do not wane during chronic treatment (in contrast to its impact on food intake), which is a prerequisite for any compound to be used as a drug to treat the metabolic syndrome.

Limitations of Our Studies

The most important limitation of our studies may be the fact that we used only one dose of PYY3–36. Circulating PYY3–36 levels in fasting conditions remained below the level of detec-
tion (≤1 pg/μl) during continuous treatment and rose to 3.7 ± 0.8 pg/μl ~1 h after subcutaneous injection. During hyperinsulinemia (3–3.5 h after injection), PYY3–36 levels were undetectable by our assay in all animals. Thus, despite the fact that continuous PYY3–36 treatment did not produce measurable plasma concentrations and intermittent administration induced a merely transitory increase of circulating PYY3–36, both treatments significantly facilitated insulin-mediated glucose disposal in high-fat-fed animals. Relatively few papers report plasma PYY3–36 concentrations in rodents. Postprandial levels may be in the range of 112 pmol/l (~0.4 pg/μl) and 0.18 pg/μl in freely feeding normal weight rats and mice, respectively (3, 24), whereas fasting levels are considerably lower because PYY3–36 is primarily released in response to food intake (3, 14). Plasma PYY3–36 concentrations in high-fat-fed mice are unknown but may be significantly reduced, because obese humans have clearly diminished circulating PYY3–36 levels (2). Taken together, our data suggest that even a relatively low dose of PYY3–36 (in view of the low circulating PYY3–36 levels during treatment) can reinforce insulin action. Dose response experiments are warranted to comprehensively map the efficacy of PYY3–36 to ameliorate insulin resistance. However, even considerably higher (than our 2.5 μg/day) doses of PYY3–36 do not appear to have a greater impact on food intake and/or RQ (1, 4).

Both control and PYY-treated mice were anesthetized during the clamp procedure, which renders the groups comparable in that respect. Nevertheless, it is important to bear in mind that anesthesia can impact on insulin action. However, alternatively, mice are clamped when conscious, which is rather stressful and may therefore affect insulin action as well.

Biological and Clinical Significance

Impaired glucose tolerance and insulin resistance often accompany obesity in humans (12, 36). Also, oxidation of fatty acids is reduced in obese individuals (6), and high RQ predicts body weight gain in humans (42). Additionally, disruption of fatty acid oxidation, reflected by high RQ values, is associated with insulin resistance (22, 41). High-fat feeding has been shown to compromise mitochondrial efficiency to combust fatty acids, which may explain high (postprandial) RQ in obese humans (22, 41). Fatty acid oxidation, reflected by high RQ values, is associated with insulin resistance (22, 41). High-fat feeding has been shown to compromise mitochondrial efficiency to combust fatty acids, which may explain high (postprandial) RQ in obese individuals (6).

In conclusion, the present studies show that (acute and chronic) PYY3–36 administration redirects substrate utilization toward fatty acid oxidation and reinforces insulin action on glucose disposal in mice. These metabolic effects occur independently of the anorexic effect of PYY3–36 that wanes after 7 days of daily treatment. Thus PYY3–36 or analogous molecules may be useful tools in the treatment of insulin resistance and the metabolic syndrome.

GRANTS

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