Insulin sensitivity : modulation by the gut-brain axis

Heijboer, A.C.

Chapter 5

PYY_{3-36} reinforces insulin action on glucose disposal in mice fed a high fat diet

Annemieke C. Heijboer*[1, 3], Anita M. van den Hoek*[2, 3], Eleonora P.M. Corssmit[1], Peter J. Voshol[1, 3], Johannes A. Romijn[1], Louis M. Havekes[2, 3, 4] and Hanno Pijl[1].

*both authors contributed equally

[1] Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands
[2] Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands
[3] TNO-Prevention and Health, Gaubius Laboratory, Leiden, The Netherlands
[4] Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT

Peptides YY_{3-36} (PYY_{3-36}) is released by the gut in response to nutrient ingestion. It modulates the activities of orexigenic neuropeptide Y (NPY) neurons and anorexigenic proopiomelanocortin (POMC) neurons in the hypothalamus to inhibit food intake. Because both NPY and POMC have also been shown to impact insulin action, we wondered whether PYY_{3-36} could improve insulin sensitivity. To address this question, we examined the acute effect of intravenous PYY_{3-36} on glucose and free fatty acid (FFA) flux during a hyperinsulinemic-euglycemic clamp in mice maintained on a high-fat diet for 2 weeks before the experiment. We also evaluated the effects of PYY_{3-36} infusion on glucose uptake in muscle and adipose tissue in this experimental context. Under basal conditions, none of the metabolic parameters were affected by PYY_{3-36}. Under hyperinsulinemic conditions, glucose disposal was significantly increased in PYY_{3-36}-infused compared with vehicle-infused mice (103.8 ± 10.9 *vs.* 76.1 ± 11.4 µmol/min/kg, respectively; P=0.001). Accordingly, glucose uptake in muscle and adipose tissue was greater in PYY_{3-36}-treated animals, although the difference with controls did not reach statistical significance in adipose tissue (muscle: 2.1 ± 0.5 *vs.* 1.5 ± 0.5 µmol/ g tissue, P=0.049; adipose tissue: 0.8 ± 0.4 *vs.* 0.4 ± 0.3 µmol/ g tissue; P=0.08). In contrast, PYY_{3-36} did not impact insulin action on endogenous glucose production or FFA metabolism. These data indicate that PYY_{3-36} reinforces insulin action on glucose disposal in mice fed a high-fat diet, through a mechanism that is independent of food intake and body weight. In contrast, it leaves glucose production and lipid flux largely unaffected in this experimental context.

INTRODUCTION

Peptide YY (PYY) belongs to a family of peptides that is critically involved in the regulation of appetite. It is synthesized in specialized cells (L-cells) that are found primarily in the distal gastrointestinal tract. Circulating PYY levels rise within 15 minutes after a meal in proportion to the amount of calories ingested and remain elevated for ~6 h (1). The two natural forms of this peptide, PYY_{1-36} and PYY_{3-36}, have opposing effects on food intake (2): PYY_{1-36} stimulates appetite whereas PYY_{3-36} (the major circulating form) inhibits feeding (3-5).

PYY_{3-36} reduces food intake by acting on appetite regulation centers in the hypothalamus (3;6). Specifically, PYY_{3-36} is an agonist of the presynaptic neuropeptide Y (NPY) Y2 receptor, which inhibits NPY neuronal activity in the arcuate nucleus and thereby activates adjacent proopiomelanocortin (POMC) neurons (3). In addition to their critical role in the control of feeding, both NPY and POMC affect insulin action. Intracerebroventricular infusion of NPY induces hyperinsulinemia and insulin resistance in rats (7;8). In contrast, central administration of α-melanocyte stimulating hormone, a splice-product of the POMC peptide, enhances insulin sensitivity (9). In view of the fact that PYY_{3-36} inhibits NPY neuronal activity and activates that of POMC, we wondered whether it could improve insulin sensitivity “directly” (i.e., through a mechanism independent of food intake and body weight). To address this question, we infused PYY_{3-36} intravenously and quantified its acute effects on glucose and fatty acid flux during a hyperinsulinemic-euglycemic clamp in mice without access to food during the experiment.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice were housed in a temperature-controlled room on a 12-h light/dark cycle and were fed a high-fat diet (43 energy% fat derived from bovine lard) with free access to water for 2 weeks to induce insulin resistance. 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.

Hyperinsulinemic-euglycemic clamp

A total of 36 mice were fasted overnight with food withdrawn at 5:00 P.M. the day before the study. The next day, hyperinsulinemic-euglycemic clamps were performed as described earlier (10). PYY or vehicle was administered as a primed (0.15 µg)-continuous (0.25 µg/h) intravenous infusion during the whole experiment (basal and hyperinsulinemic period). In one series of experiments, glucose turnover was quantified, and in another series, free fatty acid (FFA) turnover was determined. First, basal rates of glucose or FFA turnover were measured by giving a primed-continuous infusion of ^{3}H-glucose (prime: 0.7 µCi; continuous: 1.2 µCi/h; Amersham, Little Chalfont, U.K.) or ^{14}C-palmitate (prime: 1.8 µCi; continuous: 3 µCi/h; Amersham), respectively, for 80 min. Subsequently, insulin was administered in a primed (4.1 mU)-continuous (6.8 mU/h) intravenous infusion for 2-3 h to attain steady-state circulating insulin levels of ~6 ng/ml. A variable infusion of 12.5% D-glucose was used to maintain euglycemia, measured at 10 min intervals via tail bleeding (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, FFA, and insulin concentrations and [^{3}H]glucose and [^{14}C]palmitate specific activities.

To assess insulin-mediated glucose uptake in individual tissues, 2-deoxy-D-[^{3}H] glucose (2-DG; Amersham) was administered as a bolus (1µCi) 40 minutes before the end of the clamp experiments in which FFA turnover was measured. 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 FFAs were determined using commercially available kits (Instruchemie, Delfzijl, The Netherlands, and Wako, Neuss, Germany). Plasma insulin and PYY_{3-36} concentrations were measured by a mouse insulin enzyme-linked immunoabsorbant assay and PYY_{3-36} radioimmunoassay (Alpco, Windham, NH, and Phoenix Pharmaceuticals, Belmont, CA). Total plasma ^{3}H-glucose was determined in 7.5 µl plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water. Total plasma ^{14}C-palmitate was determined in 7.5 µl plasma after extraction of lipids by a modification of Bligh and Dyer's (11) method. Briefly, 7.5 µl plasma was dried and resolved in 100 µl water. Then 1.1 ml demi-water and 4.5 ml methanol:chloroform (2:1) were added and mixed thoroughly, after which 1.5 ml chloroform was added and mixed, and, finally, 1.5 ml demi-water was added and mixed. After centrifugation, the chloroform layer was

collected, and the FFA fraction was separated from other lipid components by thin-layer chromatography (TLC) on silica gel plates.

Tissue analysis

For determination of tissue 2-DG uptake, the homogenate of muscle and adipose tissue was boiled, and the supernatant was subjected to an ion-exchange column to separate 2-DG-6-phosphate from 2-DG as described previously (10;12;13).

Calculations

Turnover rates of glucose and FFAs (µmol/min/kg) 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 ^{3}H-glucose or ^{14}C-palmitate (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 was calculated from tissue 2-DG content, corrected for plasma specific activity and expressed as micromoles per gram of tissue.

Statistical analysis

Differences between groups were determined by Mann-Whitney's non-parametric test for two independent samples. $P < 0.05$ was considered statistically significant. All values shown represent mean ± SD.

RESULTS

Animals

Mice were 16-18 weeks old during the experiments. Body weight was 23.3 ± 1.2 g in the control group and 22.8 ± 1.4 g in the PYY group.

Plasma parameters

Plasma glucose, FFA, insulin, and PYY_{3-36} concentrations in basal and hyperinsulinemic conditions are shown in table 1. In the basal state, plasma parameters did not differ between PYY- and vehicle-infused animals, except for the plasma PYY_{3-36} concentration. Under steady-state clamp conditions, plasma insulin and glucose concentrations were similar in both groups. Hyperinsulinemia suppressed FFA levels to a similar extent in PYY- and vehicle-infused mice.

Table 1. Plasma parameters in overnight fasted mice that received an i.v.-infusion of PYY or vehicle under basal or hyperinsulinemic conditions. Values represent mean ± SD for at least 7 mice per group.

	Basal		Hyperinsulinemic	
	Vehicle	**PYY**	**Vehicle**	**PYY**
Glucose (mmol/l)	**4.9 ± 0.6**	**5.0 ± 0.6**	**9.0 ± 0.9**	**9.2 ± 2.0**
FFA (mmol/l)	**0.9 ± 0.2**	**1.0 ± 0.2**	**0.4 ± 0.1**	**0.5 ± 0.1**
Insulin (ng/ml)	**0.3 ± 0.1**	**0.3 ± 0.1**	**5.2 ± 2.9**	**6.7 ± 2.7**
PYY_{3-36} (pg/µl)	**< 1**	**3.8 ± 0.7**	**< 1**	**3.2 ± 0.7**

Data are the means ± SD for at least seven mice per group

Glucose turnover

Under basal conditions, glucose disposal was similar in PYY- and vehicle-infused mice (39,5 ± 10.9 vs. 45.9 ± 12.6 µmol/min/kg, respectively; P=0.19) (Figure 1a). The rate of glucose infusion necessary to maintain euglycemia during insulin infusion was significantly higher in PYY-infused mice than in vehicle-infused animals (89.1 ± 7.1 vs. 50.9 ± 13.6 µmol/min/kg, P=0.001), indicating that intravenous PYY_{3-36} administration acutely enhances insulin sensitivity. Hyperinsulinemia increased glucose disposal in both groups. However, the disposal rate was significantly higher in PYY-infused animals compared with vehicle-infused controls (103.8 ± 10.9 vs. 76.1 ± 11.4 µmol/min/kg, respectively; P=0.001) (Figure 1a). In contrast, EGP, which was similar in PYY- and vehicle-treated mice under basal conditions, was

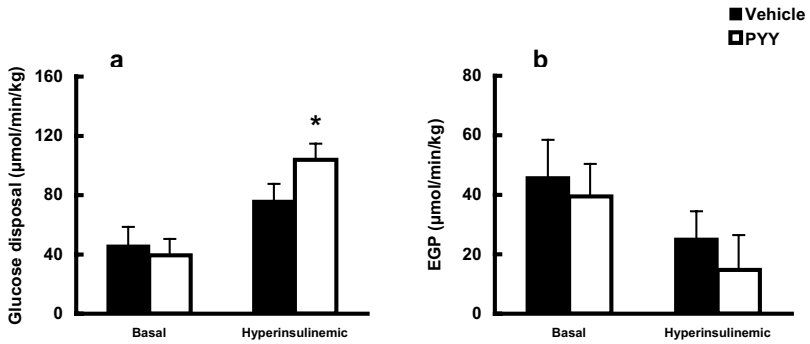

Fig. 1. Glucose disposal (a) and endogenous glucose production (b) in overnight-fasted mice before (basal) and during (hyperinsulinemic) a hyperinsulinemic-euglycemic clamp. Before and during insulin infusion the animals received an intravenous infusion of PYY or vehicle. Values represent mean ± SD for at least seven mice per group. *P<0.01 vs. vehicle.

suppressed by insulin to the same extent in both groups (by 62 ± 29 vs. 42 ± 18% from basal, respectively; P=0.30) (Figure 1b).

Tissue-specific glucose uptake

Insulin-mediated 2-DG uptake in muscle and adipose tissue was greater in PYY-treated animals, but the difference with controls did not reach statistical significance in adipose tissue (muscle: 2.1 ± 0.5 vs. 1.5 ± 0.5 µmol/g tissue, P=0.049; adipose tissue: 0.8 ± 0.4 vs. 0.4 ± 0.3 µmol/g tissue; P=0.08) (Figure 2)

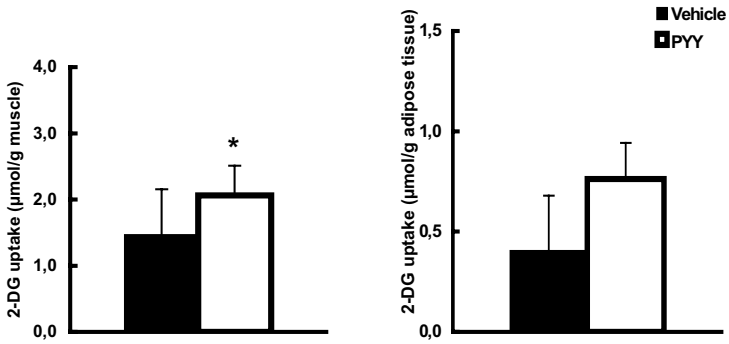

Fig. 2. Muscle-specific (a) and adipose tissue-specific (b) glucose uptake under hyperinsulinemic conditions in overnight-fasted mice that received an intravenous infusion of PYY or vehicle. Values represent mean ± SD for at least seven mice per group. *P<0.05 vs. vehicle.

FFA turnover

Basal FFA turnover (38.0 ± 14.2 vs. 42.3 ± 9.9 µmol/min/kg) was not different between PYY- and vehicle-infused animals and was suppressed to a similar extent by insulin in both groups (22.4 ± 12.3 vs. 21.3 ± 10.9 µmol/min/kg in PYY- and vehicle-infused animals, respectively) (Figure 3).

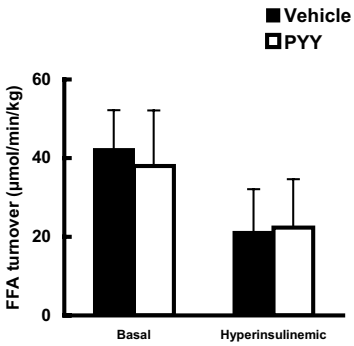

Fig. 3. Free fatty acids (FFA) turnover in overnight fasted mice before (basal) and during (hyperinsulinemic) a hyperinsulinemic-euglycemic clamp. Before and during insulin infusion the animals received an intravenous infusion of PYY or vehicle. Values represent mean ± SD for at least nine mice per group.

DISCUSSION

Here we show that intravenous PYY_{3-36} administration acutely reinforces insulin action on glucose disposal in overnight-fasted mice maintained on a high-fat diet. In contrast, PYY_{3-36} does not appear to impact the effect of insulin on EGP or FFA metabolism in this experimental context.

PYY_{3-36} clearly enhanced insulin-induced glucose disposal as determined by tracer dilution methodology. Accordingly, 2-DG uptake in muscle and adipose tissue under hyperinsulinemic conditions was higher during PYY_{3-36} infusion, albeit the difference with control attained statistical significance only for muscle. In contrast, PYY_{3-36} did not significantly impact the capacity of insulin to inhibit EGP. Insulin action on FFA metabolism was not affected by PYY_{3-36}, either, as indicated by similar fatty acid turnover rates during hyperinsulinemia in PYY_{3-36}- and saline-infused animals. In light of the current experimental context, these data suggest that PYY_{3-36} reinforces the impact of insulin on glucose disposal through a mechanism that is independent of food intake and body weight. In contrast, it appears to leave insulin action on glucose production and FFA turnover largely unaffected.

The plasma PYY_{3-36} concentration rose to 3.2 ± 0.7 pg/µl in response to PYY infusion. Relatively few studies have looked at the physiology of circulating PYY_{3-36} in rodents. According to a recent paper by Batterham et al.(3), postprandial PYY_{3-36} concentrations amount to 112 pmol/l (~0.4 pg/µl) in freely feeding rats. In a study by Lee et al (14), plasma PYY levels in fasting mice were 0.18 pg/µl, which accords with our data (table 1). We are not aware of any study measuring postprandial PYY_{3-36} concentrations in mice. Thus, the dose of PYY_{3-36} we administered may have induced supraphysiological PYY_{3-36} levels. Therefore, our data do not allow a definitive inference as to whether the postprandial rise of circulating PYY_{3-36} can reinforce insulin action.

Postprandial PYY_{3-36} release is decreased in obese compared with lean subjects, and circulating levels correlate negatively with BMI. Conversely, intravenous PYY_{3-36} infusion significantly reduces food intake in humans (15), and repeated administration of PYY_{3-36} attenuates weight gain in rodents (3). These findings suggest that diminished PYY_{3-36} release may contribute to the pathogenesis of obesity in animals and humans. The observations presented here allow us to hypothesize that low circulating PYY_{3-36} levels may also compromise insulin action in obese subjects. Moreover, perhaps even more important, the data suggest that PYY_{3-36} or synthetic analogs of this peptide may be useful tools in the clinical management of obesity and insulin resistance.

It remains to be established whether PYY_{3-36} acts through hypothalamic neural circuits (by analogy with the mechanism guiding its effects on appetite) to enhance insulin-mediated glucose disposal. Because PYY_{3-36} is a high-affinity agonist to the Y2 receptor (2) and the distribution of this receptor subtype is largely confined to the central nervous system (particularly the arcuate nucleus of the hypothalamus) (16), it is most likely that PYY_{3-36} modulates insulin action by activation of Y2 receptors in the brain. As alluded to earlier, Y2 receptor signaling inhibits NPY neuronal activity in the arcuate nucleus of the hypothalamus and thereby activates adjacent POMC neurons (3). Hypothalamic overexpression of NPY induces obesity and insulin resistance in mice (7;8), whereas activation of melanocortin receptor subtypes 3 and 4 in the brain enhances insulin sensitivity (9). Thus, the present data are in keeping with the contention that PYY_{3-36} modulates insulin action via hypothalamic Y2 receptor. The downstream mechanisms that actuate the effects of hypothalamic neuronal circuits on muscle and adipose tissue remain to be fully elucidated. At this point, vagotomy was shown to prevent the hyperinsulinemic effects of NPY, which suggests that the autonomic nervous system may be involved (17). Also, adrenalectomy prevents the obesity syndrome produced by chronic central NPY infusion and reverses the obese phenotype in leptin-deficient *ob/ob* mice (18;19), indicating that the pituitary-adrenal ensemble may also serve as a second messenger. Thus, neural and/or endocrine mechanistic routes may convey signals from hypothalamic nuclei to peripheral tissues to orchestrate energy balance and fuel flux. It is conceivable that similar routes partake in the control of insulin action by PYY_{3-36}.

In conclusion, PYY_{3-36} reinforces insulin action in mice maintained on a high-fat diet through a mechanism that is independent of food intake and body weight. In this context, PYY_{3-36} appears to predominantly impact insulin-mediated glucose disposal, whereas it leaves insulin action on glucose production largely unaffected. These novel data suggest that PYY_{3-36} or synthetic analogues of this peptide may be valuable assets to our armamentarium of drugs designed to battle insulin resistance and type 2 diabetes mellitus.

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