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Insulin sensitivity : modulation by the gut-brain axis

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Chapter 6

Chronic PYY₃₋₃₆ treatment ameliorates insulin resistance in C57BL6-mice on a high fat diet

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Submitted

ABSTRACT

Aims/hypothesis. PYY₃₋₃₆ is a gut-derived hormone, that acts on hypothalamic nuclei to modulate energy metabolism. We recently showed, that PYY₃₋₃₆ acutely reinforces insulin action on glucose disposal in insulin resistant mice. However the long-term effects of PYY₃₋₃₆ on insulin sensitivity are still unknown.

Methods. To address this question, we examined the effects of chronic PYY₃₋₃₆ administration (2.5 µg/day s.c. for 7 days) on glucose turnover during a hyperinsulinemic-euglycemic clamp in C57BL6 mice maintained on a high fat diet for 16 weeks before the experiment. In addition, metabolic efficacy of continuous vs. intermittent administration of PYY₃₋₃₆ was evaluated.

Results. Under hyperinsulinemic conditions, glucose disposal was significantly increased in PYY₃₋₃₆ treated mice vs. vehicle-treated mice (78.8 ± 13.3 vs. 63.4 ± 15.5 µmol/min/kg, respectively, $P=0.012$). Tissue specific glucose uptake was significantly increased in adipose tissue (0.5 ± 0.2 vs. 0.2 ± 0.1 µmol/ g tissue; $P=0.006$), but not in muscle (2.2 ± 1.4 vs. 1.6 ± 0.8 µmol/ g tissue for PYY₃₋₃₆ and vehicle-treated animals, respectively, $P=0.38$) of PYY₃₋₃₆ treated animals. In contrast, insulin action on endogenous glucose production was not significantly affected. Furthermore, none of these metabolic parameters were affected by the mode of PYY₃₋₃₆ administration (continuous or intermittent).

Conclusions/interpretation. Chronic PYY₃₋₃₆ administration enhances the ability of insulin to promote glucose disposal, whereas it does not significantly affect endogenous glucose production in C57BL6 mice maintained on a high fat diet for 16 weeks. In addition, this study shows that continuous and intermittent administration are equally effective in this respect.

INTRODUCTION

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 high-density lipoprotein (HDL) cholesterol [1-3]. Insulin resistance may underlie the majority of these pathologies [4] and therapies that effectively reinforce insulin action may therefore ameliorate the risk profile of metabolic syndrome patients [5;6].

Diet-induced obese, insulin resistant C57BL6-mice have increased levels of neuropeptide Y (NPY) and decreased levels of pro-opiomelanocortin (POMC) in hypothalamic nuclei [7-9]. These features of hypothalamic neural circuits may be involved in the pathogenesis of the metabolic syndrome, as intracerebroventricular (icv) administration of NPY or antagonists of POMC induce insulin resistance [10-13]. Therefore, antagonists of NPY and/or agonists of POMC signalling may be useful tools in the clinical management of this syndrome. Peptide YY₃₋₃₆ (PYY₃₋₃₆) 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 NPY neuronal activity and thereby activates adjacent POMC neurons [14;15]. We recently found that PYY₃₋₃₆ administration acutely reinforces insulin action on glucose disposal through a mechanism that is independent of food intake and body weight [16]. This finding suggests that PYY₃₋₃₆ may be used as a therapeutic tool in the clinical management of insulin resistance and the metabolic syndrome. However, the metabolic effects of long-term PYY₃₋₃₆ administration are currently unknown, and waning of early impact may occur during chronic treatment through down regulation of receptor expression or function [17;18]. Therefore, the aim of this study was to investigate the long-term effects of PYY₃₋₃₆ on insulin action by administering PYY₃₋₃₆ subcutaneously for 7 days in mice fed a high-fat diet, and quantifying the effects on glucose production and disposal during a hyperinsulinemic euglycemic clamp study. As the physiology of PYY₃₋₃₆ entails intermittent release in response to food intake, we also examined whether continuous and intermittent administration of PYY₃₋₃₆ impact glucose metabolism differentially in this experimental context.

MATERIAL AND METHODS

Animals

Male C57BL6 mice were housed in a temperature-controlled room on a 12-hour light-dark cycle and were fed a high fat diet (43 energy% fat derived from bovine lard) with free access to water for 16 weeks to induce insulin resistance. After 15 weeks of high fat diet, 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 (n = 15) or PYY₃₋₃₆ (2.5 µg/day, n = 5) infusion via the osmotic minipump at a rate of 0.5 µl/h for 7 days. In addition, daily subcutaneous injections (50 µl at 09.00 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 (n = 8) or PYY₃₋₃₆ (n = 7) by injection. Thus, glucose kinetics were determined in 2 experimental groups: 1) receiving saline and 2) receiving PYY₃₋₃₆, where PYY₃₋₃₆ was administered continuously by minipump or intermittently by daily subcutaneous injection. 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

Mice were fasted overnight with food withdrawn at 05.00 pm the day prior to the study. The next day, hyperinsulinemic euglycemic clamps were performed as described earlier [19]. 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, U.K.) for 80 min. Subsequently, insulin was administered in a primed (4.1 mU) continuous (6.8 mU/h) i.v. infusion for 2 to 3 hours to attain steady state circulating insulin levels of ~3.5 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 BV, Vianen, The Netherlands). Blood samples (75 µl) were taken during the basal period (after 60 and 80 minutes) and during the clamp period (when glucose levels were stable and 20 and 40 minutes later) for determination of plasma glucose, non-esterified fatty acids (NEFA), 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, Little Chalfont, UK) was administered as a bolus (1µCi), 40 minutes before the end of the clamp experiments. At the end of the clamp, mice were sacrificed and muscle and adipose tissue were isolated and frozen in liquid nitrogen for subsequent analysis.

Analytical procedures

Plasma levels of glucose and NEFA were determined using commercially available kits (Instruchemie, Delfzijl, The Netherlands and Wako, Neuss, Germany). Plasma insulin and PYY₃₋₃₆ concentrations were measured by a mouse insulin ELISA and PYY₃₋₃₆ RIA (Mercodia, Uppsala, Sweden; Phoenix pharmaceuticals, Belmont, CA, USA; sensitivity of 1 pg/µl for the PYY₃₋₃₆ RIA). Total plasma ¹⁴C-glucose was determined in 7.5 µl 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 was subjected to an ion-exchange column to separate 2-DG-6-P from 2-DG as described previously [19-21].

Calculations

Turnover rates of glucose ($\mu\text{mol}/\text{min}/\text{kg}$) 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 ^{14}C -glucose (dpm/ μmol). The ratio was corrected for body weight. 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 μmol per gram of tissue.

Statistical analysis

Differences between groups were determined by Mann-Whitney non-parametric test for 2 independent samples. A P-value < 0.05 was considered statistically significant. All values shown represent means $\pm$ SD.

RESULTS

Animals

Body weight did not differ between PYY₃₋₃₆ and vehicle-infused animals (after 7 days of PYY₃₋₃₆/saline administration: 28.0 ± 3.7 gram in the PYY₃₋₃₆ group and 28.3 ± 1.5 gram in the control group, $P=0.68$). Overnight food intake was measured at day 2 and day 5 of PYY₃₋₃₆/saline administration and was similar in both groups (day 2: 2.37 ± 0.68 vs. 2.32 ± 0.33 gram, $P=0.96$; day 5: 2.76 ± 0.54 vs. 2.75 ± 0.43 gram, $P=0.97$ in PYY₃₋₃₆ and vehicle-treated animals, respectively). Furthermore, body weight and overnight food intake was not different in groups receiving continuous or intermittent PYY₃₋₃₆ treatment (body weight: 29.5 ± 3.9 vs. 26.9 ± 3.4 gram, $P=0.20$; food intake day 2: 2.14 ± 0.98 vs. 2.48 ± 0.56 gram, $P=0.38$; day 5: 2.53 ± 0.69 vs. 2.92 ± 0.39 gram, $P=0.27$ for continuous and intermittent administration, respectively).

Table 1. Plasma parameters under basal or hyperinsulinemic conditions in overnight fasted mice that received PYY₃₋₃₆ (n=12) or vehicle (n=8) for 7 days.

	Basal		Hyperinsulinemic	
	Vehicle	PYY ₃₋₃₆	Vehicle	PYY ₃₋₃₆
Glucose (mmol/l)	7.7 ± 1.3	8.4 ± 1.5	8.4 ± 1.2	9.4 ± 0.8
NEFA (mmol/l)	1.1 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
Insulin (ng/ml)	0.7 ± 0.3	0.7 ± 0.4	3.2 ± 0.9	3.6 ± 0.8

Data are the means ± SD. * <0.05 vs. vehicle

Plasma parameters

Plasma glucose, NEFA, and insulin concentrations in basal and hyperinsulinemic conditions are shown in table 1. Plasma glucose and insulin concentrations did not differ between vehicle and PYY₃₋₃₆ treated animals under basal and steady state clamp conditions.

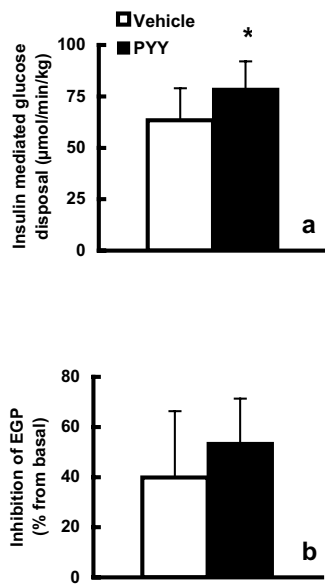


Fig 1. Insulin mediated glucose disposal (a) and inhibition of endogenous glucose production (EGP) by insulin (b) in overnight fasted mice before (basal) and during (hyperinsulinemic) a hyperinsulinemic euglycemic clamp study. Prior to the clamp experiment the animals received PYY₃₋₃₆ (n=12) or vehicle (n=8) for 7 days. Values represent the means ± SD. *P<0.05 vs. vehicle.

Furthermore, continuous and intermittent PYY₃₋₃₆ administration had similar impact on 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 (basal glucose: 9.3 ± 0.9 vs. 7.7 ± 1.5 mmol/l, P=0.048; hyperinsulinemic glucose: 9.9 ± 0.8 vs. 9.1 ± 0.6 mmol/l, P=0.073; basal insulin: 0.9 ± 0.4 vs. 0.5 ± 0.3 ng/ml, P=0.073; hyperinsulinemic insulin: 3.9 ± 1.0 vs. 3.4 ± 0.6 ng/ml, P=0.43). Plasma NEFA concentrations were slightly, but significantly, lower in PYY₃₋₃₆ treated mice in basal (P=0.025) and steady state clamp (P=0.031) conditions, where continuous and intermittent PYY₃₋₃₆ administration did not have differential effects (basal NEFA: 0.9 ± 0.3 vs. 0.9 ± 0.1 mmol/l, respectively, P=0.76; hyperinsulinemic NEFA: 0.5 ± 0.1 vs. 0.4 ± 0.1 mmol/l, respectively, P=0.073). 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 intermittent PYY₃₋₃₆ administration (3.7 ± 0.8 pg/μl).

Glucose turnover

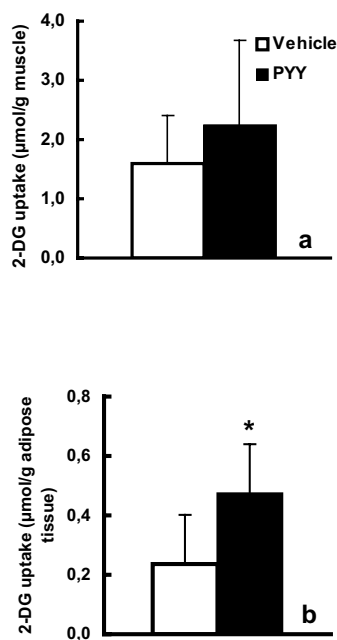


Fig 2. Muscle-specific (a) and adipose tissue-specific (b) glucose uptake under hyperinsulinemic conditions in overnight fasted mice that received PYY₃₋₃₆ (n=11) or vehicle (n=7) for 7 days. Values represent the means $\pm$ SD. *P<0.05 vs. vehicle.

In basal conditions, glucose disposal was similar in PYY₃₋₃₆ and vehicle-treated mice (52.0 ± 10.5 vs. 50.4 ± 10.4 μmol/min/kg, respectively, $P=0.68$). The rate of glucose infusion necessary to maintain euglycemia during insulin infusion was significantly higher in PYY₃₋₃₆ treated mice than in vehicle-treated animals (54.0 ± 11.4 vs. 33.4 ± 11.6 μmol/min/kg, $P=0.000$), indicating that chronic PYY₃₋₃₆ administration enhances whole body insulin sensitivity. Continuous and intermittent administration of PYY₃₋₃₆ had similar effects on the glucose infusion rate (54.7 ± 9.2 vs. 53.6 ± 10.2 μmol/min/kg, respectively, $P=0.27$). Hyperinsulinemia increased glucose disposal in both groups. However, the disposal rate was significantly higher in PYY₃₋₃₆ treated animals compared with vehicle-treated controls (78.8 ± 13.3 vs. 63.4 ± 15.5 μmol/min/kg, respectively, $P=0.012$, Figure 1a) and was similar in animals treated by continuous and intermittent administration (81.2 ± 13.8 vs. 77.1 ± 13.7 μmol/min/kg, respectively, $P=0.64$). Endogenous glucose production was similar in PYY₃₋₃₆ and vehicle-treated mice in basal conditions and was suppressed

by insulin to the same extent in both groups (by 54 ± 18 vs. $40 \pm 26\%$ from basal in PYY₃₋₃₆ vs. vehicle treated groups, respectively; $P=0.27$, Figure 1b), where percent inhibition did not differ between animals receiving continuous or intermittent PYY₃₋₃₆ treatment. (52 ± 25 vs. $55 \pm 12\%$ from basal, respectively, $P=0.53$).

Tissue-specific glucose uptake

Insulin-mediated 2-deoxy-glucose uptake was measured in muscle and adipose tissue. In muscle, 2-deoxy-glucose was similar in both groups (2.2 ± 1.4 vs. 1.6 ± 0.8 μmol/g tissue for PYY₃₋₃₆ and vehicle-treated animals, respectively, $P=0.38$). In adipose tissue 2-deoxy-glucose uptake was significantly increased in PYY₃₋₃₆ treated animals compared with vehicle treated mice (0.5 ± 0.2 vs. 0.2 ± 0.1 μmol/g tissue; $P=0.006$, Figure 2).

DISCUSSION

Here we show that chronic PYY₃₋₃₆ administration improves whole body insulin sensitivity of glucose metabolism in C57BL6 mice maintained on a high fat diet for 16 weeks. 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. In addition, this study documents that continuous and intermittent administration of PYY₃₋₃₆ reinforce insulin action to a similar extent.

These data corroborate our previous findings, which unveil similar acute effects of PYY₃₋₃₆ administration on insulin action [16], and support the emerging concept of neural circuits controlling fuel flux, independent of their impact on food intake and body weight. In addition, our data indicate that the effects of PYY₃₋₃₆ on glucose metabolism do not wane during chronic treatment, which suggests that this peptide may be a novel asset in the battle against insulin resistance and the metabolic syndrome.

Although PYY₃₋₃₆ enhanced insulin-induced glucose disposal, it did not significantly affect the ability of insulin to inhibit endogenous glucose production. Nonetheless, we can not exclude the possibility that the experimental group size may have limited the statistical power necessary to detect a subtle influence of PYY₃₋₃₆ on hepatic glucose metabolism. Alternatively, PYY₃₋₃₆ exerts differential, tissue specific, effects on insulin action.

The mechanism by which PYY₃₋₃₆ affects insulin-mediated glucose metabolism remains to be elucidated. Perhaps, PYY₃₋₃₆ modulates insulin action via the hypothalamic Y2 receptor, in analogy with the mechanism guiding its effects on appetite. Y2-receptor mediated inhibition of NPY and stimulation of POMC neuronal activity by PYY₃₋₃₆ potentially reinforces insulin action on glucose metabolism indeed [10;11;13].

Circulating PYY₃₋₃₆ levels in fasting conditions remained below the level of detection (< 1 pg/μl) during continuous treatment, and rose to 3.7 ± 0.8 pg/μl approximately one hour after i.p injection. During hyperinsulinemia (3-3.5 hours after injection), PYY₃₋₃₆ levels were undetectable by our assay in all animals. Thus, in spite of 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 [14;22], whereas fasting levels are considerably lower, as PYY₃₋₃₆ is primarily released in response to food intake [14;23]. Plasma PYY₃₋₃₆ concentrations in high fat fed mice are unknown, but may be significantly reduced, as obese humans have clearly diminished circulating PYY₃₋₃₆ levels [24]. 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. Further dose-response experiments are warranted to evaluate the potential efficacy of PYY₃₋₃₆ in the treatment of the metabolic syndrome.

Food intake and body weight were not affected by PYY₃₋₃₆ administration in our study. These findings agree with data from Challis *et al.*, indicating that 7 days of PYY₃₋₃₆ administration did not affect food intake and body weight in POMC^{-/-} and wild type mice [25]. In contrast, Batterham *et al.* reported that PYY₃₋₃₆ acutely inhibits food intake [14], an observation that could not be reproduced by Tschöp and coworkers [26;27]. To take this issue further, we compared the acute effects of a single intraperitoneal (2.5 µg) injection of PYY₃₋₃₆ (n = 8) or vehicle (n = 8) at 09.00 am on food intake in our animals, and found that cumulative food intake in 4 hours after injection was significantly inhibited by 21% in overnight fasted mice (P=0.028), whereas subsequent feeding over 24 hours was not affected by PYY₃₋₃₆. These data suggest that this dose of PYY₃₋₃₆ has a short-term inhibitory impact on food intake in overnight fasted C57BL6 mice, whereas consumption over 24 hours is not affected, probably as a result of a rebound compensatory increase of appetite [14;15].

In conclusion, the present study shows that chronic PYY₃₋₃₆ administration reinforces insulin action on glucose disposal in mice maintained on a high fat diet, whereas it also tends to enhance the ability of insulin to suppress endogenous glucose production. These observations suggest that PYY₃₋₃₆ or potential analogues may be a useful treatment for insulin resistance and the metabolic syndrome.

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