Effects of peripheral administration of PYY$_{3–36}$ on feed intake and plasma acyl-ghrelin levels in pigs

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Abstract

These studies were designed to investigate the effects of i.v. administration of peptide YY$_{3–36}$ (PYY$_{3–36}$) on feed intake, acyl-ghrelin, and GH levels in castrated male pigs. Feed intake levels were evaluated during both ad libitum and fast-refed conditions, and plasma hormone responses were evaluated during fasting. During ad libitum feeding, i.v. injection of PYY$_{3–36}$ (30 $\mu$g/kg body weight, BW) significantly reduced feed intake levels within 3 h post-treatment. In the fast-refed condition, both single bolus injection (30 $\mu$g/kg BW) and i.v. infusion (0·25 $\mu$g/kg BW per min) of PYY$_{3–36}$ suppressed feed intake levels 1 h post-treatment. Duration of the elevation of plasma PYY levels induced by i.v. injection of porcine PYY$_{3–36}$ in ad libitum-fed pigs was longer compared with the values of fasted or fast-refed pigs. In the infusion study, the elevation of plasma PYY levels was maintained throughout the infusion period and values were reduced less than half at 15 min after termination of infusion. These results showed that the anorexigenic short-term effect of PYY$_{3–36}$ treatment corresponds to its half-life. However, i.v. PYY$_{3–36}$ injection did not influence plasma acyl-ghrelin levels. On the other hand, single bolus injection of PYY$_{3–36}$ increased plasma GH levels 30 min after treatment. Similar to previous findings in other mammalian species, the results of these studies show that PYY$_{3–36}$ can reduce feed intake levels; in particular, the effect is potent and acute in pigs. Furthermore, basal plasma PYY levels were higher in ad libitum-fed pigs than in fasted pigs suggesting that circulating PYY$_{3–36}$ levels influence satiety and contribute to the termination of feed intake in pigs.


Introduction

Peptide YY (PYY), a 36-amino-acid peptide that shares a common tertiary structure with neuropeptide Y (NPY) and pancreatic polypeptide (PP) (Berglund et al. 2003), was initially isolated from porcine intestine (Tatemoto & Mutt 1980). This peptide exists in two major circulating forms, PYY$_{1–36}$ or PYY$_{3–36}$ (Grandt et al. 1994). PYY$_{3–36}$ is a 34-amino-acid peptide, which is derived from PYY$_{1–36}$ via cleavage from the N-terminal by dipeptidyl peptidase IV. PYY$_{3–36}$ is able to cross the blood–brain barrier (Nonaka et al. 2003) and peripheral administration of the peptide has been demonstrated to reduce feed intake (Batterham et al. 2002). PYY$_{3–36}$ influences feeding by inhibition of NPY release and the activation of pro-opiomelanocortin (POMC) neurons via the NPY Y2 receptor (Y2-R) of the arcuate nucleus (ARC) in rats and mice. Furthermore, chronic i.p. injection of PYY$_{3–36}$ to rats has been shown to reduce weight gain. The anorexigenic effect of PYY$_{3–36}$ has also been demonstrated during i.v. infusion studies conducted in normal (Batterham et al. 2002), obese and lean humans (Batterham et al. 2003). Furthermore, i.m. PYY$_{3–36}$ injection to rhesus monkeys similarly reduced feed intake (Moran et al. 2005). In pigs and humans, PYY is primarily produced in the distal small intestine and the large bowel, and plasma PYY levels rise substantially in response to eating (Adrian et al. 1985, 1987).

On the other hand, ghrelin is a peptide that is primarily produced in the oxyntic mucosa of the stomach of pigs (Govoni et al. 2005). In contrast to PYY$_{3–36}$, ghrelin is known to have stimulatory effects on food intake in rats (Nakazato et al. 2001) and humans (Wren et al. 2001). In pigs, plasma PYY levels increase after meal intake (Adrian et al. 1987), plasma ghrelin levels rise during fasting (Salfen et al. 2003, Govoni et al. 2005). In addition, ghrelin infusion has also been shown to increase weight gain and plasma GH levels in pigs (Salfen et al. 2004). Previous studies in rats and humans suggest that more or less PYY$_{3–36}$ and ghrelin may interact closely to regulate feed intake and energy expenditure. In pigs, so far, there are no reports on the anorexigenic and metabolic effects of PYY$_{3–36}$. Therefore, in this study, we investigated the effects of PYY$_{3–36}$ on feed intake and plasma PYY levels in pigs during ad libitum and fast-refed conditions. Furthermore, we examined whether PYY$_{3–36}$ treatment could affect plasma acyl-ghrelin and growth hormone (GH) levels during fasting conditions.
Materials and Methods

Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine. Crossbred (Large White × Landrace × Duroc) castrated male pigs were housed in individual pens with rubber slotted floors. Commercial diet (crude protein 16%, crude fat 2-5%, crude fiber 5%, and crude ash 7%) was available ad libitum and was supplied twice a day at 0900 and 1700 h and it showed that feed intake levels in all pigs were consistent prior to the start of experiment. Water was accessible all the time. Animals were anesthetized and indwelling catheters were inserted into the extra-jugular vein 3 days prior to treatment (Phung et al. 2000). To facilitate infusion of peptide and blood sampling at the same time, another catheter heter was inserted in the contralateral extra-jugular vein. Patency of the catheter was returned to normal.

Peptides and method of administration

Porcine PYY$_{3-36}$ (AKPEAPGEDASPEELRHYLNLTRQRY-NH$_2$) and [Cys-0]-porcine PYY$_{4-36}$ were synthesized by solid-phase peptide synthesis method using the Fmoc (9-fluorenylmethoxycarbonyl) protection strategy with Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu), Fmoc-Cys(Trt)-OH, Fmoc-Glu(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)-OH, Boc-Ala-OH, and Rink Amide MBHA resin and purified by reverse phase HPLC (TSKgel ODS-120A; TOSOH, linear gradient of 0-60% CH$_3$CN). The purified peptides were lyophilized and stored at −30°C. PYY$_{3-36}$ was dissolved in 5 ml sterile saline to come up with a dose of 30 µg/kg body weight (BW) for single bolus i.v. injection. For infusion, PYY$_{3-36}$ (0.25 µg/kg BW per min) was prepared just prior to administration via dilution in 120 ml saline and infused over a period of 120 min.

Blood sampling

The blood samples (5 ml each) were collected at −30, 0, 15, 30, 60, 90, 120, 150, and 180 min relative to injection time, and at −30, 0, 30, 60, 90, 120, 135, 150, and 180 min relative to the start of the infusion period. Blood samples were immediately transferred to centrifuge tubes containing heparin (10 IU/ml) and chilled on ice. Plasma samples were obtained after centrifugation at 3000 r.p.m. and 4°C for 30 min, and stored at −30°C until plasma assay. For acyl ghrelin assay, 50 µl 1 M HCl/ml plasma was added to each sample, and stored at −30°C until plasma acyl ghrelin assay.

Feed intake measurement

In experiments 1 and 2, pre-weighed feed was given immediately after i.v. injection of PYY$_{3-36}$. In experiment 3, pre-weighed feed was given after termination of infusion. Feed intake was measured at 1, 3, 6, 12, and 24 h after feed was given. The residual feed was returned to be given after measuring. Feed intake levels were calculated after monitoring the spillage of feed and expressed as feed consumed (g) per kg BW to minimize the large individual differences.

Experimental design

Experiment 1: Effect of i.v. injection of PYY$_{3-36}$ on feed intake in ad libitum-fed pigs

Five crossbred castrated pigs (initiation study BW ± S.E.M., 71.4 ± 2.2 kg) were continuously fed ad libitum prior to PYY$_{3-36}$ treatment. Pigs were given a single bolus i.v. injection of sterile saline or PYY$_{3-36}$ (30 µg/kg BW) at 0900 h. After injection, pre-weighed feed was given immediately, and intake levels were monitored until 24 h post-treatment. Blood sampling and feed intake measurements were carried out as mentioned earlier. Animals were repeatedly used in a randomized 2 × 2 crossover design and treatments were carried out at 2-day intervals.

Experiment 2: Effect of i.v. injection of PYY$_{3-36}$ on feed intake during overnight fast-refed condition

In this study, six overnight-fasted pigs (48.5 ± 1.3 kg BW) were initially used. Following PYY$_{3-36}$ (30 µg/kg BW) injection, pre-weighed feed was immediately given. Blood sampling and feed intake measurements were carried out as mentioned earlier. Animals were repeatedly used in a randomized 2 × 2 crossover design and treatments were carried out at 2-day intervals.

Experiment 3: Effect of 2 h i.v. infusion of PYY$_{3-36}$ on feed intake in fast-refed pigs

To compare the effects of PYY$_{3-36}$ according to the difference of administration procedure, we investigated the effects of i.v. infusion on feed intake and plasma PYY levels in fast-refed pigs. Five pigs (85.3 ± 2.9 kg BW) were studied in a randomized 2 × 2 crossover design and treatments were carried out at 2-day intervals. PYY$_{3-36}$ (0.25 µg/kg BW per min) or saline were infused for 2 h (total 30 µg/kg BW per 120 min) during fasting. After infusion, pre-weighed feed was immediately given. Blood sampling and feed intake measurements were carried out as mentioned earlier.

Experiment 4: Effects of i.v. injection of PYY$_{3-36}$ on plasma acyl-ghrelin and GH levels after an overnight fast

To study the feeding-unrelated effect of PYY$_{3-36}$ on plasma parameters, five pigs (81.2 ± 5.5 kg BW) were fasted overnight for ~16 h prior to the single bolus i.v. injections of either sterile saline or PYY$_{3-36}$ (30 µg/kg BW) at 0900 h. Blood was collected as mentioned earlier. These pigs were
Plasma assays

Plasma PYY, acyl-ghrelin, and GH concentrations were measured by double-antibody RIA procedures. For measurement of porcine PYY, polyclonal antibody for [Cys-0]-porcine PYY3–36 was generated in rabbit by a similar method as in the previously described study (ThidarMyint et al. 2006) and used at final dilution of 1/20 000. Synthesized porcine PYY3–36 was radioiodinated by the chloramine-T method (Tai et al. 1975) and purified by HPLC. Initially, PYY3–36 standard or plasma samples were incubated with anti-porcine PYY antiserum diluted in assay buffer (0·05 M phosphosaline containing 1% BSA, pH 7·4) and 125I-porcine PYY3–36 tracer (6000 c.p.m./100 µl assay buffer containing 1% normal rabbit serum). After 24-h incubation period, precipitating reagent containing goat anti-rabbit IgG was added to the reaction mixture and incubated for 30 min. The supernatant was discarded after centrifugation and radioactivity of the pellet was counted with a gamma counter (ARC 1000, Aloka, Japan). The antibody used in this assay system recognizes porcine PYY3–36 and PYY3–16 (422704; Phoenix Pharmaceuticals, Inc., Belmont, USA), but it does not recognize porcine NPY (491028; Peptide Institute, Inc. Japan), human PYY1–36 (422778; Phoenix), and human PP (421041; Phoenix) (Fig. 1). Displacement curve of 125I-labeled porcine PYY3–36 with 25, 50, 75, and 100 µl porcine plasma was parallel to the standard curve. Recovery of known amounts of porcine PYY3–36 added to a pool of porcine plasma was 96·4±% of the added amount. The average assay sensitivity for all studies was 0·1 ng/ml. The mean intra-assay coefficient of variance was 9±4%.

Plasma ghrelin levels were measured as previously described (ThidarMyint et al. 2006) using porcine acyl-ghrelin as standard. Displacement curve of 125I-labeled porcine acyl-ghrelin with porcine plasma was parallel to the standard curve. Recovery of known amounts of porcine acyl-ghrelin added to a pool of porcine plasma was 96·0±% of the added amount. The average assay sensitivity for all studies was 16±4 pg/ml. The mean intra-assay coefficient of variance was 6·2%.

Plasma GH concentrations were measured as previously described by Inoue et al. (2005). Porcine GH antiserum (AFP422801) and porcine GH (AFP10864B) were obtained from Dr A F Parlow (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, USA). The average recovery of the added amount was 107±7% and the mean intra-assay coefficient of variance was 12±6%.

Statistical analysis

All data are presented as means±S.E.M. Feed intake was analyzed using one-way ANOVA. Hormonal data for individual time points between control and PYY3–36-treated groups were compared using Student’s paired t-test. All analyses were performed using SPSS for Windows, version 10.0.0 (SPSS, Chicago, IL, USA). P<0·05 was considered significant.

Results

Experiment 1: Effect of i.v. injection of PYY3–36 on feed intake in ad libitum-fed pigs

Basal plasma PYY levels in the control group did not change throughout the experiment (2·2±0·2 ng/ml). Plasma PYY levels were elevated after injection of porcine PYY3–36 (Fig. 2A) and were maintained at higher levels until 120 min compared with the values of saline-injected group.

Cumulative feed intake levels in ad libitum-fed pigs following i.v. injection of either saline or PYY3–36 (30 µg/kg BW) is shown in Fig. 2B. Single bolus injection of PYY3–36 significantly reduced cumulative feed intake within 3-h post-treatment. Cumulative feed intake levels at 1 and 3 h after PYY3–36 treatment were reduced by 68·5±18·0% (P<0·01) and 44·2±18·6% (P<0·05) respectively, as compared with the saline-treated group. Injection of PYY3–36 also tended to reduce the feed intake until 24 h post-injection in ad libitum-fed pigs (40·9±13·9, 16·1±1·9, and 16·3±7·8% for 6, 12, and 24 h cumulative feed intake compared with saline injection group), although no significant difference was observed.
Experiment 2: Effect of i.v. injection of PYY3–36 on feed intake during overnight fast-refed condition

Averaged basal plasma PYY level was 0.7 ± 0.1 ng/ml in fast-refed pigs. Similar to the ad libitum-fed condition, elevated plasma PYY levels responsive to porcine PYY3–36 injection decreased within 60 min post-treatment (Fig. 3A).

Cumulative feed intake levels for fast-refed pigs i.v. injected with saline or PYY3–36 (30 μg/kg BW) is shown in Fig. 3B. Administration of PYY3–36 significantly reduced feed intake 1-h post-treatment as compared with saline group (12.9 ± 1.9 vs 20.6 ± 1.3 g/kg BW, P<0.01). Thereafter, feed intake levels returned to similar values as the saline groups.

Experiment 3: Effect of 2 h i.v. infusion of PYY3–36 on feed intake in fast-refed pigs

Saline infusion did not modify the plasma PYY levels throughout the study (Fig. 4A). In the PYY3–36 group, plasma PYY levels were maintained at high levels compared with the values of the saline group (27.3 ± 2.1 vs 9.6 ± 0.1 ng/ml, P<0.001) during infusion and were reduced to less than half the values at 15 min after termination of PYY3–36 infusion (135 min).

Pre-infusion with PYY3–36 for 2 h prior to feeding significantly reduced feed intake only at 1-h post-treatment as compared with the saline group (5.8 ± 1.7 vs 14.1 ± 1.6 g/kg BW, P<0.01; Fig. 4B).

Experiment 4: Effects of i.v. injection of PYY3–36 on plasma acyl-ghrelin and GH levels after an overnight fast

As shown in Fig. 5A, plasma PYY levels were elevated by porcine PYY3–36 injection and decreased to values below half of the peak level at 30 min post-treatment. Plasma acyl-ghrelin levels did not change after injection of saline or PYY3–36 (Fig. 5B). Plasma GH levels significantly increased 30 min after PYY3–36 injection compared with the saline-injected group (7.8 ± 2.1 vs 3.0 ± 0.6 ng/ml, P<0.05; Fig. 5C).
Comparison of average plasma PYY levels between the ad libitum-fed and fasting conditions are presented in Fig. 6. Plasma PYY levels were significantly higher in ad libitum-fed conditions than in the fasting condition (2.2 ± 0.2 vs 0.8 ± 0.1 ng/ml, *P < 0.001).

Discussion

Feeding is regulated mainly by NPY, an orexigenic agent, and POMC, an anorexigenic agent in hypothalamic ARC. PYY3–36 inhibits feeding by decreasing hypothalamic NPY expression and increasing hypothalamic POMC expression (Batterham et al. 2002). To date, the anorexigenic effect of PYY3–36 has been reported in rodents (Batterham et al. 2002), humans (Batterham et al. 2002, 2003), and rhesus monkeys (Moran et al. 2005). However, the effect of PYY3–36 on feed intake has not yet been confirmed in pigs. In this study, we investigated the effect of PYY3–36 on feed intake during ad libitum-fed and fast-refed conditions in pigs. Single bolus i.v. injection of PYY3–36 (30 μg/kg BW) inhibited feed intake during both the feeding conditions. In ad libitum-fed pigs, single bolus injection of PYY3–36 significantly inhibited feed intake.

Figure 4 Plasma PYY levels (A) and cumulative feed intake levels (B) in overnight fast-refed pigs after 2 h i.v. infusion of saline (○) and open bars) or PYY3–36 (●) and solid bars). Pre-weighed feed was given immediately after termination of infusion. Values are the mean ± s.e.m. of five animals. Horizontal bar indicates the time of infusion. Asterisks indicate significant difference between treatments (*P < 0.05, †P < 0.01).

Figure 5 Plasma PYY (A), acyl-ghrelin (B), and GH levels (C) in fasted pigs after i.v. injection of saline (○) or PYY3–36 (30 μg/kg BW, ●) at 0 min. Values are the mean ± s.e.m. of five animals. Asterisks denote significant difference between treatments (*P < 0.05).
suppressed feed intake over a period of 3 h, while during fasted-refed condition, significant reduction in feed intake was observed only within 1-h post-treatment. Similarly, 2 h i.v. infusion of PYY3–36 (0.25 μg/kg BW per min) also reduced feed intake 1 h after the termination of infusion in fasted-refed pigs. These results suggest that the anorexigenic effect of PYY3–36 in pigs is potent and acute. This finding supports the results reported by Scott et al. (2005) in rats. These short-term effects of PYY3–36 on inhibition of feed intake may correspond to the short half-life of i.v. administered PYY3–36. Our data showed that the half-life of i.v. administered PYY3–36 is <30 min post-injection and 15 min after termination of i.v. infusion. The half-life of the i.v. administered PYY3–36 has been reported as 13 min in mouse (Nonaka et al. 2003) and 19 min in rabbit (Sileno et al. 2006). Our anti-[Cys-0]-porcine PYY4–36 antiserum used in PYY RIA recognized a serine residue at position 18, which differs from human PYY4–36; therefore, the decrease of i.v. administered PYY3–36 levels might be due to the cleavage around position 18. Since the structural changes influence the action of PYY and receptor binding, the anorexigenic effect of PYY3–36 may become short-term.

It is well known that the post-prandial increase in plasma PYY levels is dependent on the level of calorie intake in humans; higher calorie intake induces higher concentrations of PYY for a longer duration (Adrian et al. 1985); together with evidence from that report, our data show that plasma PYY levels are elevated in the ad libitum-fed condition compared with the fasting condition in pigs. Furthermore, the proportion of PYY3–36 to total plasma PYY has been shown to increase during post-prandial periods (Grandt et al. 1994). Chelikani et al. (2005) also reported in non-food-deprived rats that the anorexigenic potency and efficacy of 15 min i.v. infusion of PYY3–36, just prior to the onset of the darkness cycle, is about three times less than a 3 h i.v. infusion at that time. These studies show that both the level and duration of increase in plasma PYY3–36 concentration may thus influence satiety and contribute to the termination of feed intake.

On the other hand, the result of our infusion study differed from those reported by Batterham et al. (2002) in humans, but are rather similar to the previous report in rats by Chelikani et al. (2005). The report in humans shows that i.v. infusion of PYY3–36 reduced calorie intake over a period of 12 h, while in the present study in pigs, PYY3–36 infusion reduced feed intake only for a period of 1 h. Although it is difficult to compare these reports with our studies because of the differences in species, experimental condition, infusion time, dose, and nutrient state, it can be considered for some of the reasons. First, in the human study, subjects could freely select the meal because of a buffet meal style. Furthermore, food intake levels were measured by calorie intake but not by weight. Secondly, the involvement of the experimental stress and/or length of acclimatization period might be the possible reason; i.e. injection of PYY3–36 to 16 h fasted and non-acclimated mice did not reduce feed intake, but significantly reduced feed intake in acclimated mice (Halatchev et al. 2004). All together, it is suggestive that experimental protocol/stress/acclimatization and other factors influence the anorexigenic potency of PYY3–36.

Recently, two conflicting studies reported on the effects of PYY3–36 on plasma acyl-ghrelin concentrations. Batterham et al. (2003) reported that i.v. infusion of PYY3–36 to obese and lean subjects suppressed caloric intake and significantly decreased plasma total ghrelin levels, while Adams et al. (2004) reported that i.p. injection of PYY3–36, to overnight-fasted mice reduced feed intake significantly, but there was no effect on plasma acyl- and total ghrelin levels. In this study, bolus injection of PYY3–36 did not affect the plasma acyl-ghrelin levels in fasted pigs. Therefore, it can be considered that PYY3–36 might not be a major regulator of ghrelin secretion in pigs.

Furthermore, we investigated whether PYY3–36 affects plasma GH levels, which are known as indicators of energy expenditure, in pigs because NPY, which belongs to the PP-hold family similar to PYY (Berglund et al. 2003), has also been demonstrated to stimulate GH secretion from anterior pituitary cells of pigs, cattle, and sheep, but not rats (McMahon et al. 2001, Barb & Barrett 2005). Moreover, the effect of NPY on GH secretion is possibly mediated by NPY Y2 receptor (Y2-R) (Suzuki et al. 1996, Korbonits et al. 1999). In this study, we showed that i.v. bolus injection of PYY3–36, a potent Y2-R agonist, significantly increased plasma GH levels, and the plasma GH peak was seen 30 min after injection. Therefore, it is suggested that this effect of PYY3–36 on plasma GH levels is a direct effect on anterior pituitary cells by excessive PYY3–36 by bolus i.v. injection.

In summary, we have shown that peripheral administration of PYY3–36 in pigs can reduce feed intake. Moreover, plasma PYY levels fluctuated with the changes in energy balance leading to the suggestion that plasma PYY3–36 levels influence...
satietv and contribute to the termination of feed intake in pigs. Furthermore, the effect of PYY3–36 was potent and acute, which result is reflective of the short half-life of i.v. administered PYY3–36. Moreover, i.v. injection of PYY3–36 did not affect plasma acyl-ghrelin levels in the fasted condition indicating that at least in pigs, PYY3–36 is not a major regulator of ghrelin secretion. However, since the administration of PYY3–36 increased plasma GH levels, it is suggested that PYY3–36, apart from its influence on feed intake, may also have other metabolic functions that are crucial in the regulation of energy homeostasis in pigs.

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