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

Tetsuya Ito, Hnin ThidarMyint, Takahiro Murata, Hiroaki Inoue, Roel Mata Neyra and Hideto Kuwayama

Department of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Nishi, Inada, Obihiro 080-8555, Japan

(Requests for offprints should be addressed to H Inoue who is now at Department of Animal and Grassland Research, National Agricultural Research Center for Kyushu Okinawa Region (KONARC), 2421 Suya, Goshi 861-1192, Japan; Email: fujino@obihiro.ac.jp)

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 µg/kg body weight, BW) significantly reduced feed intake levels within 3 h post-treatment. In the fast-refed condition, both single bolus injection (30 µg/kg BW) and i.v. infusion (0.25 µg/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.

Journal of Endocrinology (2006) **191**, 113–119

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 was inserted in the contralateral extra-jugular vein. Patency of the catheter was maintained with heparinized saline. All experiments were carried out after feed intake levels of the catheterized pigs returned to normal.

Peptides and method of administration

Porcine PYY₃₋₃₆ (AKPEAPGEDASPEELSRYYASLR-HYLNLVTRQRY-NH₂) and [Cys-0]-porcine PYY₄₋₃₆ 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-Gln(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% CH₃CN). The purified peptides were lyophilized and stored at –30 °C. PYY₃₋₃₆ 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, PYY₃₋₃₆ (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 PYY₃₋₃₆. 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 PYY₃₋₃₆ 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 PYY₃₋₃₆ treatment. Pigs were given a single bolus i.v. injection of sterile saline or PYY₃₋₃₆ (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 PYY₃₋₃₆ 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 PYY₃₋₃₆ (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 PYY₃₋₃₆ on feed intake in fast-refed pigs To compare the effects of PYY₃₋₃₆ 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. PYY₃₋₃₆ (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 PYY₃₋₃₆ on plasma acyl-ghrelin and GH levels after an overnight fast To study the feeding-unrelated effect of PYY₃₋₃₆ 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 PYY₃₋₃₆ (30 µg/kg BW) at 0900 h. Blood was collected as mentioned earlier. These pigs were

repeatedly used in a randomized 2×2 crossover design and treatments were carried out at 2-day intervals.

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 PYY₄₋₃₆ 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 PYY₁₋₃₆ (422704; Phoenix Pharmaceuticals, Inc., Belmont, USA), but it does not recognize porcine NPY (491028; Peptide Institute, Inc. Japan), human PYY₁₋₃₆ (422787; 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 $68.5 \pm 18.0\%$ (*P*<0.01) and $44.2 \pm 18.6\%$ (*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 $16.3 \pm 7.8\%$ for 6, 12, and 24 h cumulative feed intake compared with saline injection group), although no significant difference was observed.

Figure 1 Standard RIA curve for porcine PYY. Inhibition of ¹²⁵I-labeled porcine PYY₃₋₃₆ binding to rabbit anti-[Cys-0]-porcine PYY₄₋₃₆ by serial dilution of porcine PYY₃₋₃₆ (○), porcine PYY₁₋₃₆ (□), porcine NPY (△), human PYY₁₋₃₆ (■), and human PP (◆). Inhibition of ¹²⁵I-labeled porcine PYY₃₋₃₆ binding to antisera by serial dilution of pooled porcine plasma (●) parallel to the curve was obtained using control porcine PYY₃₋₃₆ as standard. Each point is the mean of triplicate determinations. B/B₀, bound/bound in zero standard.

Figure 2 Plasma PYY levels (A) and cumulative feed intake levels (B) in *ad libitum*-fed pigs following i.v. injection of saline (○) and open bars) or PYY₃₋₃₆ (30 µg/kg BW, (●), and solid bars) at 0 min. Pre-weighed feed was given immediately after injection. Values are the mean ± S.E.M. of five animals. Asterisks denote significant difference between treatments (* $P < 0.05$, † $P < 0.01$).

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

Figure 3 Plasma PYY levels (A) and cumulative feed intake levels (B) in fast-refed pigs following i.v. injection of saline (○) and opened bars) or PYY₃₋₃₆ (30 µg/kg BW, (●) and closed bars) at 0 min. Pre-weighed feed was given immediately after injection. Values are the mean ± S.E.M. of six animals. Asterisks denote difference between treatments (* $P < 0.05$, † $P < 0.01$).

were maintained at high levels compared with the values of the saline group (27.3 ± 2.1 vs 0.6 ± 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).

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 $\pm$ S.E.M. of five animals. Horizontal bar indicates the time of infusion. Asterisks indicate significant difference between treatments (* $P < 0.05$, $^{\dagger}P < 0.01$).

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

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 μ g/kg BW, (●)) at 0 min. Values are the mean $\pm$ S.E.M. of five animals. Asterisks denote significant difference between treatments (* $P < 0.05$).

ad libitum-fed and fast-refed conditions in pigs. Single bolus i.v. injection of PYY₃₋₃₆ (30 μ g/kg BW) inhibited feed intake during both the feeding conditions. In *ad libitum*-fed pigs, single bolus injection of PYY₃₋₃₆ significantly

Figure 6 Comparison of average of plasma PYY levels (mean $\pm$ S.E.M.) during *ad libitum* (open bar) and fasting (solid bar) conditions in pigs. Asterisks indicate significant difference between groups (* $P < 0.001$).

suppressed feed intake over a period of 3 h, while during fast-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 μ g/kg BW per min) also reduced feed intake 1 h after the termination of infusion in fast-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.p. 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

satiety 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.

Acknowledgements

The authors gratefully acknowledge Dr A F Parlow and the National Hormone and Peptide Program for generously providing reagents and procedures for porcine GH RIA. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Adams SH, Won WB, Schonhoff SE, Leiter AB & Paterniti JR Jr 2004 Effects of peptide YY[3-36] on short-term food intake in mice are not affected by prevailing plasma ghrelin levels. *Endocrinology* **145** 4967-4975.
- Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM & Bloom SR 1985 Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* **89** 1070-1077.
- Adrian TE, Bacarese-Hamilton AJ, Smith HA, Chohan P, Manolas KJ & Bloom SR 1987 Distribution and postprandial release of porcine peptide YY. *Journal of Endocrinology* **113** 11-14.
- Barb CR & Barrett JB 2005 Neuropeptide Y modulates growth hormone but not luteinizing hormone secretion from prepuberal gilt anterior pituitary cells in culture. *Domestic Animal Endocrinology* **29** 548-555.
- Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatti MA *et al.* 2002 Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* **418** 650-654.
- Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatti MA & Bloom SR 2003 Inhibition of food intake in obese subjects by peptide YY3-36. *New England Journal of Medicine* **349** 941-948.
- Berglund MM, Hipskind PA & Gehlert DR 2003 Recent developments in our understanding of the physiological role of PP-fold peptide receptor subtypes. *Experimental Biology and Medicine* **228** 217-244.
- Chelikani PK, Haver AC & Reidelberger RD 2005 Intravenous infusion of peptide YY(3-36) potently inhibits food intake in rats. *Endocrinology* **146** 879-888.
- Govoni N, De Iasio R, Cocco C, Parmeggiani A, Galeati G, Pagotto U, Brancica C, Spinaci M, Tamanini C, Pasquali R *et al.* 2005 Gastric immunolocalization and plasma profiles of acyl-ghrelin in fasted and fasted-refed prepuberal gilts. *Journal of Endocrinology* **186** 505-513.
- Grandt D, Schimiczek M, Beglinger C, Layer P, Goebell H, Eysselein VE & Reeve JR Jr 1994 Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. *Regulatory Peptides* **51** 151-159.
- Halatchev IG, Ellacott KL, Fan W & Cone RD 2004 Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. *Endocrinology* **145** 2585-2590.
- Inoue H, Watanuki M, Hnin TM, Ito T, Kuwayama H & Hidari H 2005 Effects of fasting and refeeding on plasma concentrations of leptin, ghrelin, insulin, growth hormone and metabolites in swine. *Animal Science Journal* **76** 367-374.
- Korbonits M, Little JA, Forsling ML, Tringali G, Costa A, Navarra P, Trainer PJ & Grossman AB 1999 The effect of growth hormone secretagogues and neuropeptide Y on hypothalamic hormone release from acute rat hypothalamic explants. *Journal of Neuroendocrinology* **11** 521-528.
- McMahon CD, Radcliff RP, Lookingland KJ & Tucker HA 2001 Neuroregulation of growth hormone secretion in domestic animals. *Domestic Animal Endocrinology* **20** 65-87.
- Moran TH, Smedh U, Kinzig KP, Scott KA, Knipp S & Ladenheim EE 2005 Peptide YY(3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **288** R384-R388.
- Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K & Matsukura S 2001 A role for ghrelin in the central regulation of feeding. *Nature* **409** 194-198.
- Nonaka N, Shioda S, Niehoff ML & Banks WA 2003 Characterization of blood-brain barrier permeability to PYY3-36 in the mouse. *Journal of Pharmacology and Experimental Therapeutics* **306** 948-953.
- Phung LT, Inoue H, Nou V, Lee HG, Vega RA, Matsunaga N, Hidaka S, Kuwayama H & Hidari H 2000 The effects of growth hormone-releasing peptide-2 (GHRP-2) on the release of growth hormone and growth performance in swine. *Domestic Animal Endocrinology* **18** 279-291.
- Salfer BE, Carroll JA & Keisler DH 2003 Endocrine responses to short-term feed deprivation in weanling pigs. *Journal of Endocrinology* **178** 541-551.
- Salfer BE, Carroll JA, Keisler DH & Strauch TA 2004 Effects of exogenous ghrelin on feed intake, weight gain, behavior, and endocrine responses in weanling pigs. *Journal of Animal Science* **82** 1957-1966.
- Scott V, Kimura N, Stark JA & Luckman SM 2005 Intravenous peptide YY3-36 and Y2 receptor antagonism in the rat: effects on feeding behaviour. *Journal of Neuroendocrinology* **17** 452-457.
- Sileno AP, Brandt GC, Spann BM & Quay SC 2006 Lower mean weight after 14 days intravenous administration peptide YY(3-36) (PYY(3-36)) in rabbits. *International Journal of Obesity* **30** 68-72.
- Suzuki N, Okada K, Minami S & Wakabayashi I 1996 Inhibitory effect of neuropeptide Y on growth hormone secretion in rats is mediated by both Y1- and Y2-receptor subtypes and abolished after anterolateral deafferentation of the medial basal hypothalamus. *Regulatory Peptides* **65** 145-151.
- Tai HH, Korsch B & Chey WY 1975 Preparation of ¹²⁵I-labeled secretin of high specific radioactivity. *Analytical Biochemistry* **69** 34-42.
- Tatemoto K & Mutt V 1980 Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature* **285** 417-418.
- ThidarMyint H, Yoshida H, Ito T & Kuwayama H 2006 Dose-dependent response of plasma ghrelin and growth hormone concentrations to bovine ghrelin in Holstein heifers. *Journal of Endocrinology* **189** 655-664.
- Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatti MA & Bloom SR 2001 Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and Metabolism* **86** 5992-5995.

Received 7 March 2006

Received in final form 12 July 2006

Accepted 21 July 2006

Made available online as an Accepted Preprint
7 August 2006