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ARTICLE

Effects of dipeptidyl peptidase IV on the satiety actions of peptide YY

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Abstract
Aims/hypothesis Dipeptidyl peptidase IV (DP IV) inhibitors are currently being developed to prolong the biological activity of insulinotropic peptides as a novel approach in the treatment of diabetes. We hypothesised that DP IV inhibition could attenuate the satiety actions of peptide YY (PYY) by altering the conversion of PYY(1–36) to PYY(3–36).
Materials and methods The effects of PYY delivered by osmotic mini-pumps were assessed in rats treated with a DP IV inhibitor and in a rat model deficient in DP IV.
Results Pharmacological levels of total PYY were found in the circulation after the exogenous administration of PYY (3–36). While both PYY(1–36) and PYY(3–36) reduced food intake in normal rats, PYY(1–36) was ineffective in rats deficient in DP IV. When re-fed after a 24-h fast, DP IV-deficient rats exhibited higher food intake and weight gain than normal rats. Moreover, unlike controls, there was no postprandial increase in PYY levels in DP IV-deficient rats. Despite these findings, administration of a DP IV inhibitor, Pro-boroPro, did not alter the acute anorectic effects of exogenous PYY(1–36) in normal rats. This could be the result of the protection of other appetite regulatory peptides or the generation of PYY(3–36) by remaining DP IV activity or other dipeptidyl peptidases.
Conclusions/interpretation Although DP IV inhibition with Pro-boroPro attenuated the generation of PYY(3–36), our results indicate that short-term DP IV inhibition does not eliminate the satiety actions of exogenously administered PYY(1–36) at the doses tested.

Keywords Body weight · Dipeptidyl peptidase IV · Energy balance · Food intake · Gut · Hormone · Osmotic mini-pumps · Peptide YY · Pro-boroPro

Abbreviations

DP IV	dipeptidyl peptidase IV
GIP	glucose-dependent insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
MALDI	matrix-assisted laser desorption/ionisation
PYY	peptide YY
TOF	time-of-flight

Introduction

Dipeptidyl peptidase IV (DP IV) is an aminopeptidase, which is present in both tissues and the circulation, and which cleaves dipeptides from the N-terminal region of polypeptides with a strong preference for proline or alanine at the second position [1]. For example, DP IV rapidly cleaves glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), a process that eliminates their otherwise potent insulinotropic activities [2]. Two approaches are being actively pursued to take advantage of the antidiabetic effects of these incretin

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hormones. Analogues are being developed that are resistant to inactivation by DP IV, and inhibitors of DP IV are under investigation as a means of prolonging the biological activity of endogenous GIP and GLP-1 [3–5]. Several additional endocrine and neural factors are substrates of DP IV, including neuropeptide Y, growth hormone-releasing factor, pituitary adenylate cyclase-activating polypeptide, gastrin-releasing peptide and peptide YY (PYY) [1, 6], raising the possibility that a lack of specificity of DP IV inhibitors might limit their use in treatment of diabetes.

PYY is a 36 amino acid peptide hormone released along with GLP-1 from L-cells of the distal intestine during meals [7]. DP IV cleaves the Tyr-Pro dipeptide from the N-terminus of PYY(1–36) to produce PYY(3–36), a second biologically active form of PYY [8–11]. Physiological functions regulated by PYY include glomerular filtration [12], vasoconstriction [13], gastric emptying [14], gastric acid secretion [15], secretion from the exocrine pancreas [16] and food intake [17]. Intracerebroventricular or i.p. injections [18–21] and s.c. or i.v. infusion [22–24] of PYY (3–36) in rodents, along with i.m. injections in rhesus monkeys [25] and i.v. infusion in lean and obese humans [26], have been shown to inhibit food intake. PYY(3–36) administration also inhibits weight gain in rodents [18, 23]. The satiety actions of PYY(3–36) appear to be mediated by the Y2-receptor present in the hypothalamic arcuate nucleus [17, 27]; thus satiety actions of PYY(3–36) are absent in Y2-receptor knock-out mice [18, 19]. However, there is some evidence to indicate a vagally mediated mechanism of peripheral effects of PYY [21, 28], although this remains controversial [20]. Intravenous infusion of PYY(1–36) also inhibits food intake in rats [29], probably as a result of DP IV-mediated conversion to PYY(3–36). In striking contrast, intracerebroventricular or paraventricular injections of PYY(1–36) stimulate food intake [30, 31], probably acting via hypothalamic Y1 and Y5 receptors [32].

Diminished DP IV activity would be predicted to attenuate the conversion of PYY(1–36) to PYY(3–36) and, as a result, possibly increase meal size and weight gain. Clearly, this would be an undesirable effect of DP IV inhibitor therapy, especially in subjects with type 2 diabetes, who are typically overweight. We examined the effects of PYY(1–36) and PYY(3–36) on food intake of normal and DP IV-deficient rats. The effects of a DP IV inhibitor, Pro-boroPro, on the generation of PYY(3–36) from PYY(1–36) in vitro and in vivo were also studied and we investigated the ability of exogenous PYY(1–36) to regulate food intake in the presence of Pro-boroPro. Finally, feeding pattern, food intake and periprandial PYY levels were studied in normal and DP IV-deficient rats after a 24-h fast. Our results indicate that DP IV is a key enzyme responsible for the generation of PYY(3–36), the anorectic form of PYY, yet short-term inhibition of DP IV using Pro-boroPro does not affect the satiety effects of exogenous PYY(1–36) at the doses tested.

Materials and methods

Animals

Fischer 344 rats (normal rats; 200–225 g) were purchased from Charles River Laboratories (Saint-Constant, QC, Canada). DP IV-negative Fischer 344 rats (DP IV-deficient rats; 200–225 g) were obtained from a breeding colony maintained in our animal facility. DP IV-deficient rats lack DP IV [33] and have been previously characterised [34]. In all studies, age- and weight-matched male rats were individually housed in grid cages in a 12-h light (08.00–20.00 h)/12-h darkness (20.00–08.00 h) photoperiod at 23±1°C and controlled humidity in our animal care facility. Unless otherwise stated, animals had free access to tap water and rat chow (Purina Mills, St Louis, MO, USA). Rat chow was provided on the floor of grid cages and this allowed us to collect residual food particles on a tray kept beneath cages, enabling precise monitoring of food intake. Research protocols used in this study were approved by the Animal Care Committee of our university in compliance with the Canadian Council for Animal Care guidelines.

Materials

Rat PYY(1–36) (Catalogue no. 059-03), rat PYY(3–36) (Catalogue no. 059-04) and human PYY(1–36) (Catalogue no. 059-01) were purchased from Phoenix Pharmaceuticals (Belmont, CA, USA). Human PYY(1–36) and DP IV used for the matrix-assisted laser desorption/ionisation (MALDI)-time-of-flight (TOF) mass spectrometry studies and Pro-boroPro ($C_9H_{17}BN_2O_3$; 98.6% purity) were synthesised at Probiodrug (Halle, Germany). All peptides were HPLC purified to ≥95% purity. Peptides were lot-matched when multiple vials were required in a study and were freshly prepared in 0.9% saline for each study. One-day (Model 2001D) and 14-day (Model 2ML2) Alzet osmotic mini-pumps were purchased from Durect Corporation (Cupertino, CA, USA). A total PYY ELISA kit (Catalogue no. DSL-10-33600), which detects both PYY(1–36) and PYY(3–36), was purchased from DS Labs (Webster, TX, USA) and the human PYY(3–36)-specific RIA kit was purchased from Linco Research Inc. (St Charles, MO, USA). Chocolate-flavoured Ensure (Abbott Laboratories, Saint-Laurent, QC, Canada) was purchased from a local pharmacy.

Study design

Circulating levels of total PYY during continuous infusion of PYY(3–36) in normal rats Normal rats were implanted with osmotic mini-pumps (1 day) containing saline (n=6) or 100 μg·kg body weight^{-1}·day^{-1} PYY(3–36) (n=6). Blood samples were collected at 0, 3, 9, 18 and 24 h after implantation. Plasma was separated and stored at −20°C until the PYY ELISA was conducted.

Effects of continuous infusion of PYY(3–36) for 6 days on food intake and weight gain of non-acclimatised normal rats Osmotic mini-pumps (14-day) infusing 100 μg·kg body weight^{-1}·day^{-1} PYY(3–36) (n=6) or saline (n=6) were implanted in normal rats and daily food intake and body weight were measured for 6 days after implantation. Since only a transient effect of PYY(3–36) on food intake was seen, we terminated the experiment at 6 days after implantation. To identify the effects of surgical stress and implantation of osmotic mini-pumps on food intake, we used non-acclimatised rats for this study.

In vitro effects of DP IV inhibitor on the generation of PYY (3–36) from PYY(1–36) To obtain the mass spectra of PYY (1–36) degradation by DP IV, human PYY(1–36) (62.5 μmol/l) was incubated at 37°C with 0.1 mol/l Tris buffer (pH 7.6) containing DP IV plus water or DP IV plus Pro-boroPro in a 2:2:1 ratio. Samples (10 μl) from the incubation mixture were collected at 180 min and mixed with equal volumes of 0.1% trifluoroacetic acid solution. Five microlitres of the elution solution were mixed with 5 μl MALDI matrix solution. Next, 1 μl of this mixture was transferred to a probe tip for sample crystallisation. MALDI-TOF mass spectrometry was carried out using a mass spectrometer (Voyager-DE Pro; Applied Biosystems, Darmstadt, Germany) in a positive reflector mode. The spectrometer was externally calibrated using the calmix2 standard solution of Applied Biosystems. Spectra of 300 single shots per sample were accumulated.

Effects of Pro-boroPro on plasma DP IV activity in normal rats Pro-boroPro (1,000 μg/rat) dissolved in saline (n=4), or saline alone (n=4), was injected s.c. at 0 h into normal rats. The same dose of Pro-boroPro was injected again after 12 h. Blood was collected at 0, 3, 12 and 24 h from the tail vein and plasma separated by centrifugation at 10,000 *g* for 9 min at 4°C and kept at −20°C until plasma DP IV activity was measured using a previously described protocol [2].

Role of DP IV in generating PYY(3–36) from PYY(1–36) in vivo Osmotic mini-pumps infusing 100 μg·kg body weight^{-1}·day^{-1} human PYY(1–36) were implanted into normal rats (n=6), DP IV-deficient rats (n=6) and normal rats injected with Pro-boroPro at the time of implantation (0 h). Blood samples were collected at 0 and 3 h after implantation into microfuge tubes containing DP IV inhibitor and aprotinin. Plasma was separated and stored at −20°C until an RIA specific for human PYY(3–36) was conducted. Background human PYY(3–36) immunoreactivity was subtracted from all values.

Effects of PYY(1–36) and PYY(3–36) on food intake of normal and DP IV-deficient rats Normal rats and DP IV-deficient rats were acclimatised for 7 days prior to the implantation of osmotic mini-pumps. Animals were kept in grid cages for 4 days from the day of arrival. On day 5 (day 1 of acclimatisation), animals were transferred to the procedure room on a cart, anaesthetised by inhalation of 3% isoflurane using oxygen as gaseous carrier, shaved in the area where the incision was to be made, and weighed. Once removed from the anaesthetic machine, animals quickly recovered from anaesthesia and were then returned to the animal facility. Food intake was measured by deducting the quantity of food recovered after 24-h feeding from the initial amount of food given. Acclimatisation (except shaving) was repeated daily for the next 6 days. Rats were then implanted with osmotic mini-pumps (1 day) containing saline (n=6), PYY(1–36) (100 μg·kg body weight^{-1}·day^{-1}; n=6) or PYY(3–36) (100 μg·kg body weight^{-1}·day^{-1}; n=6). The cumulative food intake for 24 h after implantation was measured.

Effects of Pro-boroPro on satiety actions of PYY in normal rats Normal rats were acclimatised as described previously and implanted with osmotic mini-pumps (1 day) infusing saline (n=6), PYY(1–36) (100 μg·kg body weight^{-1}·day^{-1}; n=6) or PYY(3–36) (100 μg·kg body weight^{-1}·day^{-1}; n=6). A fourth group received pumps infusing PYY(1–36) (100 μg·kg body weight^{-1}·day^{-1}; n=6) and two s.c. injections of 1,000 μg Pro-boroPro at 12-h intervals, while the fifth group (n=6) received two s.c. injections of 1,000 μg Pro-boroPro at 12-h intervals. In addition to the usual acclimatisation procedures, rats used in this study were acclimatised to i.p. injections of saline twice daily for 3 days prior to the experiment day.

Periprandial PYY levels in DP IV-positive and -deficient rats Normal (n=6) and DP IV-deficient (n=6) rats were fasted for 24 h from 07.00 h and gavaged with 4 ml Ensure (4.25 calories). Blood samples were collected at 0, 30, 60, 90, 150 and 210 min and plasma was immediately separated and kept at −20°C until total PYY were measured by ELISA following the manufacturer's instructions.

Food intake and weight gain of normal and DP IV-deficient rats re-fed after a 24-h fast Normal (n=6) and DP IV-

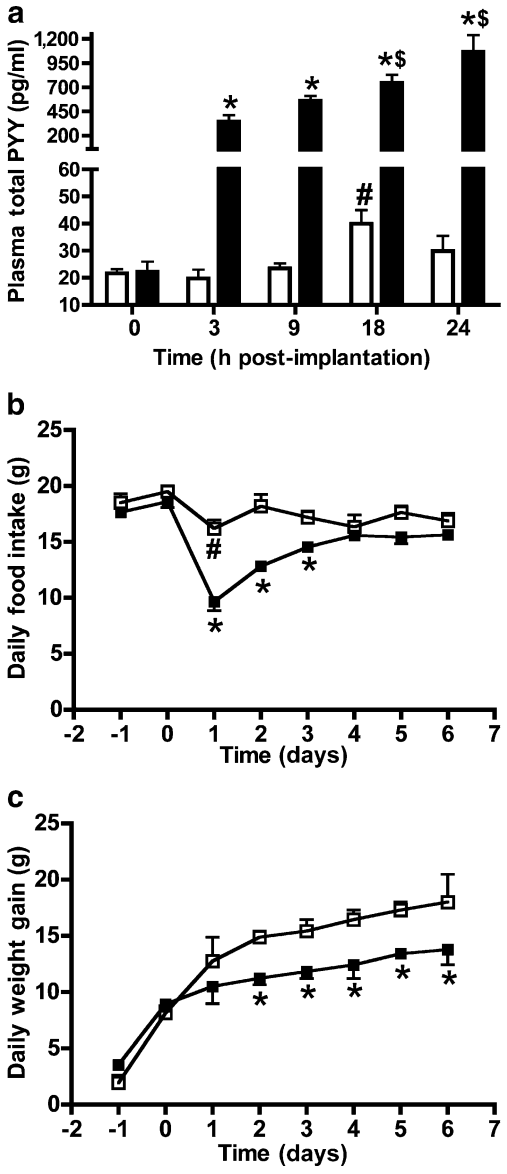

Fig. 1 **a** The 24-h profile of total PYY in the circulation in normal rats continuously infused with saline (*white bars*) or 100 μg·kg body weight^{-1}·day^{-1} PYY(3–36) (*black bars*). Data are means±SEM. n=6 rats/group. *p<0.05, saline vs treatment groups; \$$p$<0.05, PYY-treated group at 18 h (06.00 h) and 24 h (12.00 h) vs the PYY-treated group at 0 h (12.00 h) and 3 h (15.00 h); $^{\#}p$<0.05, saline-treated group at 18 h (06.00 h) vs the saline-treated group at 0, 3 and 9 h. **b**, **c** Effects of s.c. infusion of 100 μg·kg body weight^{-1}·day^{-1} PYY(3–36) (*solid squares*) or saline (*open squares*) for 6 days on daily food intake (**b**) and body weight gain (**c**) of normal rats. Data are means±SEM. n=6 rats/group. *p<0.05, saline vs treatment groups; $^{\#}p$<0.05, saline day 0 vs saline day 1

deficient (n=6) rats were fasted for 24 h from 08.00 to 20.00 h, re-fed with pre-weighed quantities of food and hourly food intake was monitored for 12 h. Cumulative food intake for 12 to 24 h and body weight gain at 24 h after re-feeding were also recorded.

Statistical analyses

All data are presented as means±SEM. Data were analysed using either a paired t-test or an ANOVA followed by a Student–Newman–Keuls test or Dunnet's multiple comparison test. p<0.05 was considered statistically significant. Graphing and statistical analyses were conducted using GraphPad Prism Version 4 (GraphPad Software Inc., San Diego, CA, USA).

Results

Circulating levels of total PYY during continuous infusion of PYY(3–36) in normal rats

Continuous infusion of PYY(3–36) caused a significant increase in total PYY levels compared with saline-treated controls at 3, 9, 18 and 24 h after implantation (Fig. 1a). Total PYY levels in PYY(3–36)-infused animals were approximately 20-, 18-, 13- and 27-fold higher than in saline-treated controls at 3, 9, 18 and 24 h after implantation, respectively. Total PYY levels in PYY-treated animals at 18 and 24 h after implantation were significantly higher than PYY levels at 3 h. In saline-treated controls, total PYY levels were significantly higher (~2-fold increase) at 18 h (06.00 h) than 0 h and all sampling points after implantation, indicating a postprandial surge in endogenous PYY in normal rats.

Effects of continuous infusion of PYY(3–36) for 6 days on food intake and body weight of non-acclimatised normal rats

Continuous infusion of PYY(3–36) for 6 days caused a transient reduction in food intake (Fig. 1b) and a prolonged reduction in weight gain (Fig. 1c) compared with the saline-treated control animals. PYY-treated animals had ~20–30% reduction in food intake on days 1 to 3 after implantation of osmotic pumps, while ~20% reduction in weight gain was observed throughout the study period. Food intake of saline-treated rats on day 1 after implantation was significantly lower than food intake on the day immediately before surgery and implantation of pumps (Fig. 1b), indicating stress-induced anorexia in these rats.

In vitro effects of DP IV inhibitor on the generation of PYY (3–36) from PYY(1–36)

Incubation of PYY(1–36) with DP IV resulted in the cleavage of PYY(1–36) to PYY(3–36) at 180 min after incubation (Fig. 2a). Co-incubation with the DP IV inhibitor, Pro-boroPro, abolished the formation of PYY(3–

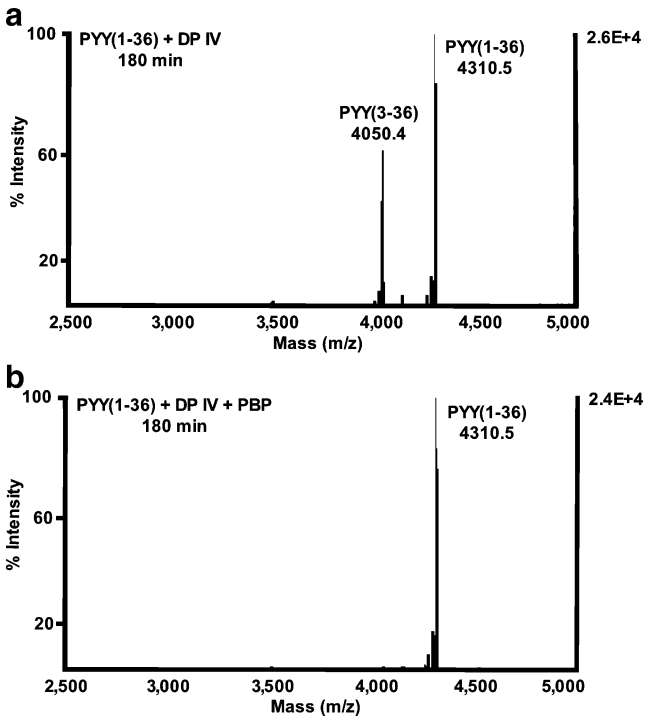

Fig. 2 MALDI-TOF mass spectrometry analysis of DP IV-catalysed hydrolysis of PYY(1–36) in the absence (**a**) or presence (**b**) of the inhibitor Pro-boroPro (*PBP*). Synthetic human PYY(1–36) ($[M+H]^+$= 4,310.4) was incubated with purified human recombinant DP IV. Samples were removed from the incubation mixture and analyte immediately crystallised with sinapinic acid as matrix and analysed by mass spectrometry. The presence of Pro-boroPro completely inhibited DP IV-induced conversion of PYY(1–36) to PYY(3–36) over a 3-h incubation period

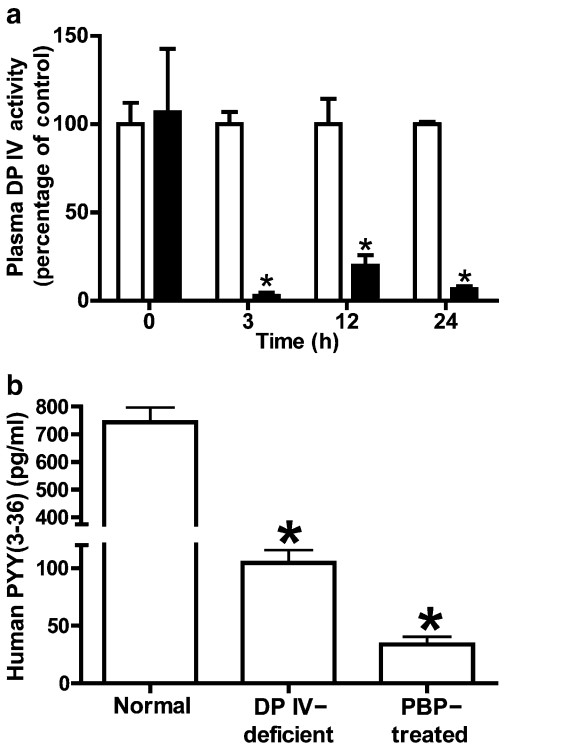

Fig. 3 **a** Effects of two s.c. injections of 1,000 μg Pro-boroPro at 12-h intervals on plasma DP IV activity in normal rats. *White bars*, saline; *black bars*, Pro-boroPro. Data are means±SEM. n=4 rats/group. *p<0.001, saline vs Pro-boroPro. **b** Circulating levels of human PYY(3–36) after the administration of human PYY(1–36) to normal and DP IV-deficient rats, and to rats treated with the DP IV inhibitor, Pro-boroPro, at 3 h after implantation of human PYY(1–36)-infusing osmotic mini-pumps. Data are means±SEM. n=6 rats/group. Overall p value for one-way ANOVA, p<0.001. *p<0.01 compared with normal rats

36) (Fig. 2b). These results indicate that Pro-boroPro is a potent DP IV inhibitor and it attenuates the DP IV-mediated conversion of PYY(1–36) to PYY(3–36).

Effects of Pro-boroPro on plasma DP IV activity in normal rats

Subcutaneous injections of Pro-boroPro caused ~97% reduction in plasma DP IV activity at 3 h after injection, ~80% reduction at 12 h after injection and ~93% reduction at 24 h after injection (Fig. 3a). There was no difference in plasma DP IV activity between study groups at 0 h (Fig. 3a). Pro-boroPro, at the dose tested, did not cause any visible symptoms of toxicity in the treatment group during the 24-h study period. Treated rats were monitored for 1 week following the study period with no findings of toxicity.

Role of DP IV in generating PYY(3–36) from PYY(1–36) in vivo

Pharmacological levels of human PYY(3–36) were found in the circulation at 3 h after infusion of human PYY(1–36) into normal rats (Fig. 3b). Detectable quantities of PYY(3–36) were also found in DP IV-deficient rats and in Pro-boroPro-treated rats (Fig. 3b). Human PYY(3–36) levels in Pro-boroPro-treated rats and DP IV-deficient rats were significantly lower than the human PYY(3–36) levels in normal rats, indicating the attenuated conversion of PYY (1–36) to PYY(3–36) due to the absence/inhibition of DP IV enzyme.

Effects of PYY(1–36) and PYY(3–36) on food intake of normal and DP IV-deficient rats

In these acclimatised rats, saline infusion did not significantly alter food intake relative to the day prior to pump implantation (17.95±0.85 vs 17.03±0.43 g/day, respectively). At a dose of 100 μg·kg body weight^{-1}·day^{-1}, both PYY (1–36) and PYY(3–36) administration significantly reduced (~20%) 24-h cumulative food intake in normal rats compared with saline-treated controls (Fig. 4a). In contrast, PYY(3–36), but not PYY(1–36), reduced food intake in DP IV-deficient rats compared with saline controls

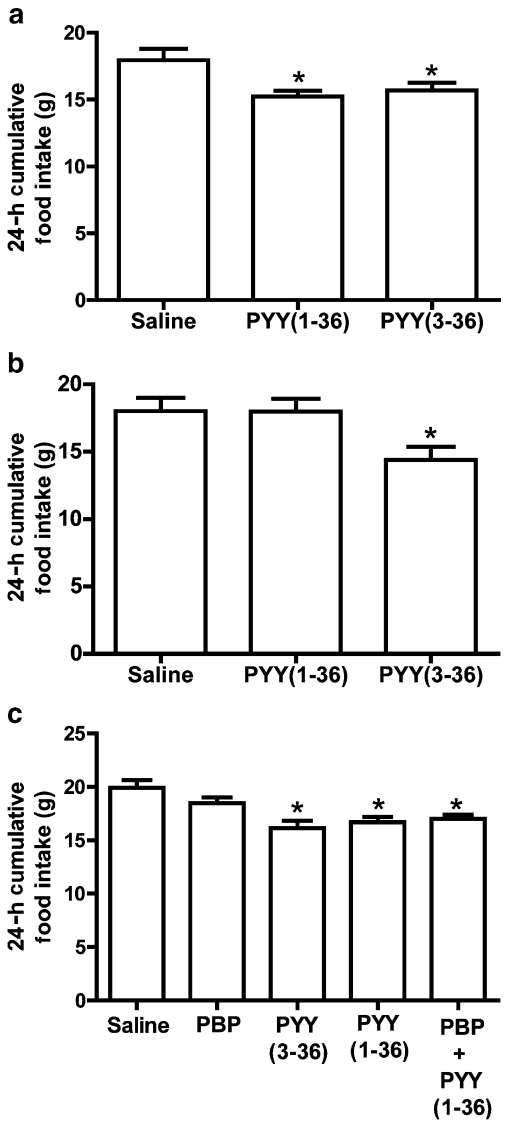

Fig. 4 Effects of s.c. infusion of 100 μg/kg body weight PYY(1–36) or PYY(3–36) for 1 day on food intake of normal rats (**a**) and DP IV-deficient rats (**b**). Data are means±SEM. n=6 rats/group. *p<0.05, saline vs treatment groups. **c** Effects of co-administration of 1,000 μg DP IV inhibitor (Pro-boroPro; *PBP*) and 100 μg/kg body weight PYY (1–36) on food intake of normal rats. In the PBP group and PBP+PYY (1–36)-treated group, two s.c. injections of PBP were given to normal rats in addition to PYY(1–36) infusion via osmotic pumps. Data are means±SEM. n=6 rats/group. Overall p value for one-way ANOVA, p<0.007. *p<0.01, saline vs treatment groups

(Fig. 4b). At a dose of 1,000 μg/kg body weight, PYY(3–36) administration reduced 24-h cumulative food intake by ~40% in normal rats (not shown).

Effects of Pro-boroPro on satiety actions of PYY in normal rats

Both PYY(3–36) and PYY(1–36) caused a significant reduction (~20%) in 24-h cumulative food intake following implantation compared with saline-treated controls (Fig. 4c). Co-administration of PYY(1–36) and Pro-boroPro also caused a significant reduction in food intake, indicating that at the doses tested Pro-boroPro has no inhibitory actions on PYY's satiety effects (Fig. 4c). A non-significant reduction in food intake was also observed in the Pro-boroPro-treated group (Fig. 4c).

Periprandial PYY levels in normal and DP IV-negative rats

DP IV-deficient rats exhibited significantly higher plasma total PYY levels than normal rats at 0 h (Fig. 5a). In normal rats, plasma total PYY levels significantly increased 30 min after re-feeding compared with basal levels (Fig. 5a) and remained significantly elevated over basal levels at 60 and 90 min after re-feeding (Fig. 5a). No periprandial changes in plasma PYY levels were found in DP IV-deficient rats.

Food intake and weight gain of normal and DP IV-negative rats re-fed after a 24-h fast

Food intake of DP IV-deficient rats at 2 h after re-feeding was significantly higher than normal rats (Fig. 5b). Cumulative food intake during the light phase (Fig. 5c) and dark phase (Fig. 5c) was also significantly higher in DP IV-deficient rats compared with normal rats. Weight gain during 24-h after re-feeding was significantly higher in DP IV-deficient rats compared with normal rats (Fig. 5d).

Discussion

DP IV inhibitors are being explored as agents to extend the biological activity of insulinotropic hormones; at the same time, they may attenuate activities of other hormones. Although the effect of DP IV inhibition on GIP and GLP-1 and its therapeutic potential in treating diabetes are well studied, few studies have examined the effects of DP IV inhibition on other peptidyl substrates of DP IV in vivo [35]. The studies discussed here analyse the effects of short-term DP IV inhibition on the satiety effects of PYY, a substrate of DP IV, as well as PYY responses and food intake in DP IV-deficient rats.

PYY is under intense study due to its ability to reduce food intake and thereby promote weight loss [18, 24]. However, several contradictory reports exist regarding the satiety effects of PYY(3–36) in rodents [22]. In our hands, continuous infusion of PYY(3–36) for 6 days caused a significant but transient reduction in food intake accompanied by a prolonged reduction in weight gain. We also found a significant reduction in the food intake of saline-treated animals on day 1 after implantation in our 6-day study. However, this effect was gone by day 2 after implantation and was absent in acclimatised rats, indicating

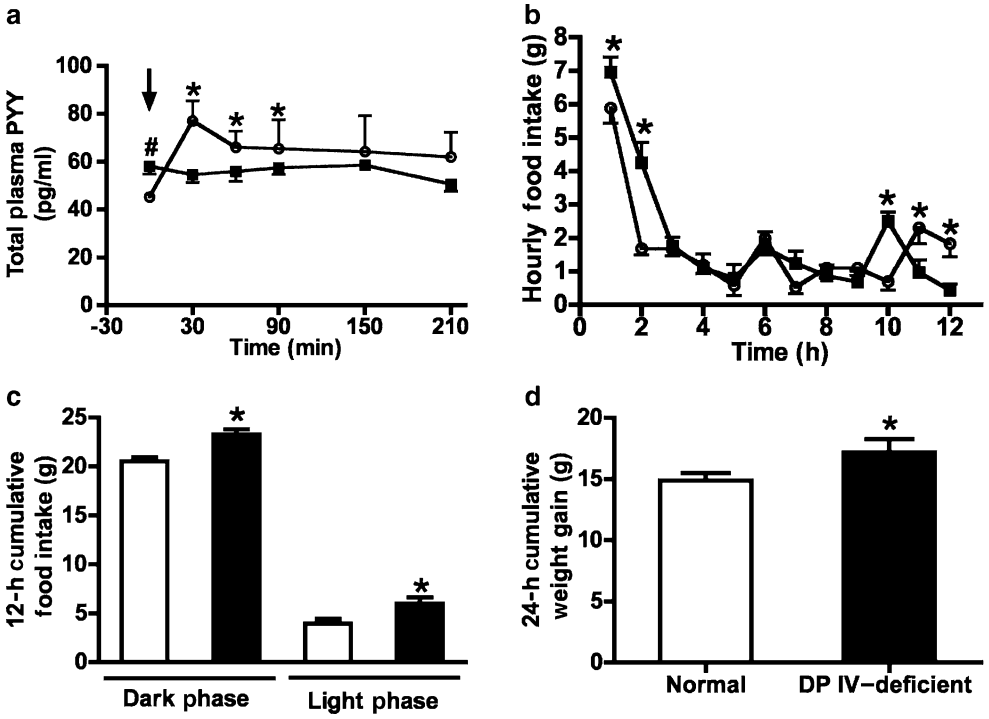

Fig. 5 **a** Periprandial plasma total PYY levels in normal and DP IV-deficient rats. *Arrow* indicates time of meal gavage. Data are means±SEM. n=6 rats/group. $^{\#}p<0.05$, PYY levels at 0 h in DP IV-deficient rats vs normal rats; $^{*}p<0.05$, PYY levels at a time-point compared with the basal PYY levels at 0 h. **b** Hourly food intake of normal and DP IV-deficient rats for 12 h following a 24-h fast; **c** cumulative food intake of normal and DP IV-deficient rats during the dark phase and light phase; and **d** cumulative weight gain after 24-h re-feeding. *Black squares/bars*, DP IV-deficient rats; *open circles/bars*, normal rats. Data are means±SEM. n=6 rats/group. $^{*}p<0.05$, DP IV-deficient rats vs normal rats

that it results from stress associated with implantation of the osmotic pump. Pumps delivering pharmacological levels of PYY(3–36) caused significantly greater and more prolonged reductions in food intake than in the saline-treated animals. This indicates that PYY(3–36) caused an anorectic effect greater than surgical stress-induced anorexia in our animals, in agreement with previously reported studies [23, 24].

Interestingly, while both PYY(1–36) and PYY(3–36) inhibited food intake of normal rats, PYY(1–36) had no effect on food intake of DP IV-deficient rats, suggesting that DP IV is required for generating the anorexigen, PYY (3–36). Our in vitro studies with purified DP IV confirm that this enzyme can indeed convert PYY(1–36) to PYY(3–36) in vitro and that Pro-boroPro, a DP IV inhibitor [36, 37], completely inhibited this conversion. Thus, inhibition of DP IV in vivo may also reduce the production of endogenous PYY(3–36).

Despite the fact that PYY(1–36) had no satiety effects in DP IV-deficient rats, suppression of DP IV activity in normal rats with Pro-boroPro did not attenuate the satiety actions of exogenous PYY(1–36). This result could be due to several reasons. Firstly, although a potent DP IV inhibitor was used, complete inhibition of DP IV in plasma was not achieved during the 24-h study period. This is indicated in our studies where at 3 h after administration of Pro-boroPro and human PYY(1–36), low but significant amounts of immunoreactive human PYY(3–36) were still produced despite the fact that approximately 97% reduction in plasma DP IV activity was achieved. Moreover, DP IV is a ubiquitous enzyme, lining the vasculature in abundance, including the microvasculature in close proximity to the PYY-producing L-cells [38]. Aside from in plasma, significant DP IV activity could remain on the vasculature such that exogenous PYY(1–36) could have been cleaved to PYY(3–36). Secondly, it is possible that enzymes other than DP IV could also generate PYY(3–36) from PYY(1–36), as PYY(3–36) is formed even in the DP IV-deficient rats. Thirdly, inhibition of DP IV would be anticipated to alter the intact levels of other regulatory peptides that could alter food intake. Indeed administration of the inhibitor alone tended to reduce food intake in our studies. One candidate hormone is GLP-1, as both central [39] and peripheral administration of GLP-1 inhibits food intake in rodents [40, 41]. GLP-1 is a substrate of DP IV and circulating levels of the active form of GLP-1 increase following the administration of DP IV inhibitors in rats [42]. Although we did not measure the circulating levels of GLP-1 levels following DP IV inhibition, such an increase in bioactive GLP-1 levels due to DP IV inhibition might also have contributed to the reduction in food intake of rats co-administered PYY(1–36) and Pro-boroPro.

Compensation can occur due to long-term DP IV deficiency, including altered levels of regulatory peptides [34] and sensitivity to their actions [35]. Cumulative food intake during the dark phase and light phase and 24-h cumulative weight gain after a 24-h fast were higher in DP IV-deficient rats than in control animals. Thus there is mild hyperphagia and increased weight gain in DP IV-deficient rats when re-fed after a 24-h fast, possibly a result of the reduced levels of the short-term satiety signal, PYY

(3–36). We also found that, in contrast to normal rats, circulating total PYY levels were elevated under basal conditions and did not increase postprandially in DP IV-deficient rats. The absent response in DP IV-deficient rats could be an adaptive/protective response to the extended half-life of PYY(1–36) in these animals. Notably, it has been previously reported that DP IV-deficient rats have lower secretion of GLP-1 and GIP compared with normal rats following an OGTT [34].

Using PYY as an example, the present study demonstrates that DP IV plays a major role in the conversion of PYY(1–36) to PYY(3–36) and DP IV inhibition attenuates this conversion. However, our results from DP IV-deficient rats suggest possible roles of other dipeptidyl peptidases in regulating the generation of PYY(1–36) to PYY(3–36). Although co-administration of PYY with a DP IV inhibitor did not change the satiety effects of PYY(1–36), the anorectic effects of PYY(1–36) were attenuated in DP IV-deficient rats and they had higher food intake and weight gain when re-fed after a 24-h fast. It remains to be determined whether acute/chronic treatment with DP IV inhibitors influences the endogenous production of PYY(3–36). Since the type of inhibitor, specificity of the inhibitor used, doses administered and the mode of administration are important factors affecting the efficacy of DP IV inhibitors, further studies using various animal models, inhibitors and peptide substrates are necessary to elucidate the effects of DP IV inhibitors on physiological processes regulated by DP IV substrates. Long-term administration of DP IV inhibitor in rats (100 days) [42] or humans (52 weeks) [43] did not alter cumulative food intake or body weight. In contrast, a 6-week therapy using GLP-1 [44] or a 28-day therapy using a GLP-1 mimetic, exenatide [45], caused significant reductions in body weight of humans. Although DP IV inhibition increases intact bioactive GLP-1 levels, and thus might be anticipated to reduce food intake, it also attenuates the conversion of endogenous PYY(1–36) to PYY(3–36), as well as potentially altering the levels of several other regulatory peptides that control food intake. Thus, it appears that the net effect of short-term DP IV inhibition on satiety is neutral.

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Duality of Interest The authors declare that they have no duality of interest relating to this manuscript.

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