Human placenta and fetal membranes contain peptide YY₁₋₃₆ and peptide YY₃₋₃₆

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Abstract

Extracts of human term amniotic, placental, and chorion/decidua tissue contained, respectively, 4.36 ± 2.79 (pmol/g wet wt; mean $\pm$ s.e.m.; $n=5$), 2.78 ± 0.5 ($n=5$) and 0.68 ± 0.68 ($n=5$) peptide YY (PYY)-like immunoreactivity. Using a specific PYY antiserum, gel filtration chromatography and reverse-phase high performance liquid chromatography (HPLC), amniotic, placental and fetal intestinal tissue extracts were demonstrated to contain PYY-like immunoreactivity consisting of equal amounts of PYY₁₋₃₆ and PYY₃₋₃₆. The presence of pancreatic polypeptide was not detected in any of the extracts.

Positive immunohistochemical staining for PYY was seen in extravillous trophoblasts in the decidual septa and fetal membranes, the syncytiotrophoblast and cytotrophoblasts, amniotic epithelial cells and in maternal decidual stromal cells. Positive staining for PYY was found at the earliest date examined (9.5 weeks) and remained present throughout pregnancy to term. PYY₁₋₃₆ and PYY₃₋₃₆ may play important roles in human pregnancy, acting via endocrine or paracrine mechanisms.

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Introduction

The pancreatic polypeptide (PP) family of peptides – PP, peptide YY (PYY) and neuropeptide Y (NPY) – is unique in that all recognized members were originally discovered by chemical means, prior to any of their biological activities having been identified. PP was discovered in a prominent chromatographic peak seen during the purification of chicken insulin (Kimmel *et al.* 1975) and, via a chemical assay which detects the presence of peptides containing the C-terminal alpha amide structure, PYY was detected in porcine intestinal extracts (Tatemoto 1982a) and NPY was detected in porcine brain extracts (Tatemoto 1982b). Initial studies localized PP and PYY to endocrine-type cells in the endocrine pancreas (Schwartz 1983) and distal intestine (Lundberg *et al.* 1982a) respectively; postprandial plasma elevations of PYY and PP levels occur (Schwartz 1983, Taylor 1989), consistent with these peptides having a humoral mechanism of action. In contrast, NPY was reported to be confined to neural structures in the peripheral, enteric and central nervous systems (Lundberg *et al.* 1982b, Furness *et al.* 1983), consistent with NPY predominantly functioning as a neurotransmitter/neuromodulator. However, our recent demonstration that PYY (McDonald *et al.* 1993, Wang

et al. 1997) rather than NPY is the predominant neuropeptide in the canine enteric nervous system, is consistent with PYY having both neurotransmitter/neuromodulator and hormonal modes of action and with PYY having a greater spectrum of activities than was initially recognized.

We recently reported that the gastrin releasing peptide (GRP), a neuropeptide in adult mammals, and apparently novel GRP-like peptides are present in the circulations and products of conception of ovine (Fraser *et al.* 1992, 1994, Xiao *et al.* 1996a) and human pregnancy (Xiao *et al.* 1996b). This surprising finding stimulated us to screen for further unexpected presences of regulatory peptides in tissues associated with human pregnancy. This study reports that, similar to GRP, PYY is also present in the products of human pregnancy. It also describes the anatomical locations and molecular forms of PYY which occur in such tissues.

Materials and Methods

Tissue collection

Within five minutes of normal term parturition, five human placentae were immediately separated into amnion, chorion/decidua, and placental villi. The

separated tissues were frozen immediately in liquid nitrogen and stored at -70°C until the time of extraction. Similarly, portions of human placentae obtained at various gestational ages were immediately fixed in 100 ml phosphate buffered saline (PBS) containing 4% para-formaldehyde–0.2% glutaraldehyde for immunohistochemistry (IHC). These tissues were obtained as waste products of pathological specimens according to the procedures outlined by the Human Ethics Committee at the University of Western Ontario. For IHC, pathology archival tissue from first trimester abortion was obtained from Dr C Goodyer (Montreal Childrens' Hospital, Montreal, Canada). Second trimester human fetal tissue was obtained following therapeutic abortion, by Dr D Hill at the Northern General Hospital, Sheffield, UK, with local ethical approval.

Tissue extraction

Thawed tissues were boiled immediately (95°C , 10 min) in 10 volumes water per unit of tissue wet weight, to inactivate proteolytic enzymes and denature structural proteins. The extraction solutions were cooled, and trifluoroacetic acid (TFA) and acetonitrile were added to make the solutions 2% and 10% (v/v) respectively. The tissues in the extraction solutions were homogenized using a hand-held (OMNI 1000) homogenizer (OMNI International, Waterbury, CT, USA) and the extraction mixture stirred for 1 h at 4°C . The extracted tissues were removed from the supernatant by centrifugation and the pellets re-extracted with 1 M acetic acid at 4°C for 1 h. The supernatants from the two extraction procedures were lyophilized. The efficiency of the extraction process for PYY was estimated as $65.9 \pm 3.9\%$ (mean $\pm$ S.E.M.; $n=6$) by the addition of radiolabelled PYY_{1–36} to heat-inactivated tissues. The tissue quantities of PYY-like immunoreactivity reported here have not been corrected.

Radioimmunoassay procedures

Radioimmunoassay (RIA) for PYY was performed using the antiserum designated LR-57. The characteristics of this antiserum, the preparation of radiolabelled PYY and the details of RIA performance have been described (McDonald *et al.* 1993). Briefly, this antiserum was raised in rabbits against porcine PYY_{1–36} (Penninsula, Belmont, CA, USA) and has no cross-reaction with PP or any structurally unrelated regulatory peptide tested, less than 0.03% cross-reaction with porcine NPY and recognizes PYY_{3–36} and PYY_{1–36} with equal potency. The final dilution of the antiserum used in the RIA was 1:400 000. The amount of synthetic porcine PYY added to the reaction mixture which decreased the initial binding of radiolabelled PYY (no unlabelled PYY added) by 10% was 2.5 fmol per assay tube, and that causing a 50% decrease in binding was 30 fmol per tube.

RIA for human PP was performed using an antiserum against PP designated 615–1054B–24A–19 (a generous gift of Dr R E Chance, Eli Lilly Laboratories, Indianapolis, IN, USA), the characteristics of which have been described in detail (Taylor *et al.* 1978). Briefly, human PP (Bachem, Torrance, CA, USA) was radiolabelled with carrier-free I¹²⁵ (Amersham, Toronto, ONT, Canada) using the Chloramine-T method as described by McDonald *et al.* (1993). After a preliminary purification of the iodination reaction mixture on a pre-washed Sep Pak C-18 cartridge (Waters Associates, Mississauga, ONT, Canada), the reaction mixture was further purified by reverse-phase HPLC using a Waters Associates apparatus and a μ BondaPak C-18 column (Waters Associates). The solvent systems used were: (A) 0.12% (v/v) TFA (Sequal grade, Pierce Chemical Company, Rockford, IL, USA) in water and (B) 0.1% (v/v) TFA in acetonitrile (Fisher Scientific, Toronto, ONT, Canada). The HPLC purification procedure employed a 35% B isocratic elution, and the radiolabelled ligand eluting at 18 min was used for RIA. Human PP was used as standard in the RIA. The antiserum was used in a final dilution of 1:2 000 000 in a total assay volume of 1 ml per tube. The buffer system employed was a 0.04 M phosphate buffer, pH 7.4, containing 0.05% sodium azide (w/v), 0.1 M sodium chloride, and 0.1% BSA (w/v). Bound and free radiolabelled ligand were separated using a charcoal–dextran technique (McDonald *et al.* 1993). The amount of synthetic human PP added to the reaction mixture which decreased the initial binding of radiolabelled PP (no unlabelled PP added) by 10% was 0.3 fmol PP per assay tube, and that causing a 50% decrease in binding was 2.5 fmol per assay tube.

Chromatographic procedures

Gel filtration chromatography was performed using 1×120 mm Sephadex G-50 (sf) columns (Pharmacia, Montréal, PQ, Canada), equilibrated and eluted with a 1 M acetic acid solution containing 0.15 M sodium chloride. The void volume (V_0) and total volume (V_d) of the columns were defined by the elution positions of Dextran Blue (Pharmacia) and carrier-free sodium I¹²⁵ (Amersham) respectively. Tissue extract samples or synthetic human PYY_{1–36}, both dissolved in the acetic acid/NaCl solution, were added in 1 ml volumes to the columns. One millilitre fractions were collected and lyophilized prior to RIA.

Reverse-phase HPLC was performed using a Beckman System Gold HPLC with Phenomenex (Phenomenex, Torrance, CA, USA) reverse-phase C-18 columns (3.9×300 mm). The solvent systems used were: (A) 0.12% (v/v) TFA in water and (B) 0.1% (v/v) TFA in acetonitrile. The solvent flow rate was 1 ml/min and elution was performed using either of two gradient elution conditions. Elution conditions I consisted of sequential linear gradient elutions of: (1) 30–44% B over 60 min,

(2) 44–65% B over 30 min, (3) 65–100% B over 10 min and (4) 100% B for 10 min. Elution conditions II consisted of sequential linear gradient elutions of: (1) 29–31% B over 60 min, (2) 31–65% B over 30 min, (3) 65–100% B over 10 min and (4) 100% B for 10 min. The reverse-phase C-18 columns were calibrated with synthetic human PYY_{1–36}, human NPY and human PP using gradient I elution conditions, and with human PYY_{1–36} and PYY_{3–36} (Bachem) using gradient II elution conditions. The elution positions of these peptides were established by monitoring UV absorption at 215 nm. Following calibration of the columns with standard peptides, the column and apparatus were thoroughly washed and blank chromatography runs were performed to ensure no carry-over of injected peptides.

Lyophilized tissue extracts with sufficient quantities of PYY-like immunoreactivity (PYY-LI) were partially purified by adsorption onto pre-washed C-18 Sep-Pak cartridges. The peptides adsorbed onto the cartridges were washed with 0.1% (v/v) TFA in water, eluted with an acetonitrile:water:TFA mixture (80%:19.9%:0.1%) and the peptides recovered from the eluates by partial evaporation of the acetonitrile and TFA under reduced pressure followed by lyophilization. Sufficient quantities of either partially purified tissue samples or standard synthetic peptides for calibration were dissolved in 1 ml solvent A and injected onto the C-18 HPLC columns. One millilitre eluate fractions were collected using gradient I elution conditions and 0.5 ml fractions were collected using gradient II elution conditions. Each collection tube contained 0.1 mg BSA (Sigma, St Louis, MO, USA) and all eluate fractions were lyophilized prior to RIA.

Three separate samples each of human amnion and placental extracts and two samples of fetal intestinal extracts were subjected to gel filtration and reverse-phase HPLC under both gradient elution conditions. The lyophilized eluate fractions obtained from the gel filtration or reverse-phase HPLC columns were reconstituted in appropriate volumes of RIA buffer prior to assay for PYY-LI.

Immunohistochemistry

Samples of chorion/decidua, amnion and placenta, fixed in a 4% paraformaldehyde–0.2% glutaraldehyde solution, were embedded in paraffin and sectioned at a thickness of 5 µm (Sangha *et al.* 1994). Samples processed and examined for PYY-LI included tissues from first trimester ($n=4$), second trimester ($n=11$), third trimester ($n=10$) and from term cases ($n=17$). Immunohistochemistry, using the antiserum LR-57 (see RIA procedures for antiserum characteristics) at dilutions between 1:500 and 1:1000, was performed by the avidin–biotin peroxidase method as previously described (Sangha *et al.* 1994). The specificity of the immunohistochemical staining was tested by pre-absorption of the antiserum with synthetic human PYY

and NPY (incubation of the antiserum with solutions of 100 µg per ml peptide overnight at 4 °C). A further control included the primary antibody being replaced with non-immune rabbit serum.

Results

Amounts and chromatographic forms of PYY-LI

All tissue extract samples were assayed at multiple dilutions and the dose–inhibition curves produced by all samples paralleled those produced by synthetic human PYY. The amounts of PYY-LI were found to be highest in the term human amnion extracts at 4.36 ± 2.79 pmoles/g tissue wet weight (mean $\pm$ s.e.m.; $n=5$). The term placental extracts contained 2.78 ± 0.50 ($n=5$) and the term decidua/chorion extracts 0.68 ± 0.68 ($n=5$) pmoles PYY-LI/g wet weight. Similar to previous findings for GRP (Fraser *et al.* 1992, Xiao *et al.* 1996b), there was variability in the amounts of measured PYY-LI between samples, especially in the decidua/chorion extracts. For comparison, the PYY-LI content in second trimester human fetal intestinal extracts was higher at 14.35 ± 3.45 pmoles/g wet weight (mean $\pm$ s.e.m.; $n=3$). There was no detectable PP-like immunoreactivity seen in any sample of the chorion/decidua, placental villi or amniotic tissue extracts ($n=5$ for each tissue).

Portrayed in Fig. 1 are the reverse-phase HPLC gradient I elution PYY-LI profiles (panels A, B, C) produced by human amnion, placental, and second trimester fetal intestinal tissue extracts. Panel D in Fig. 1 shows the PYY-LI profile produced by human amniotic tissue extracts on gel filtration chromatography. On reverse-phase HPLC using gradient I elution conditions, a dominant peak of PYY-LI was seen in all of the tissue extracts which occurred precisely at the retention time of human PYY_{1–36} (Fig. 1A,B,C). Minor amounts of PYY-LI eluting at an earlier retention time were seen in the fetal intestinal tissue extracts (Fig. 1C). No PYY-LI was seen at the elution times of NPY or PP, nor at any later elution time in any of the sample chromatograms (Fig. 1). A single peak of PYY-LI appeared at the elution volume of synthetic human PYY_{1–36} on gel filtration chromatography of human amnion (Fig. 1D), placental and second trimester fetal intestinal tissue extracts (data not shown).

Reverse-phase HPLC extracts of amnion, placental and fetal intestinal tissue under higher HPLC resolution conditions (gradient II), resolved the major PYY-LI entity into two distinct immunoreactive peaks, eluting precisely at the retention times of human PYY_{1–36} and PYY_{3–36} (Fig. 2). Approximately equal amounts of the two molecular forms were seen in all chromatographs performed. In both gradient I and II elution conditions, the fetal intestinal extracts (Figs 1C and 2C) produced slightly

Figure 1 Reverse-phase HPLC profiles of PYY-LI present in tissue extracts of (A) amnion, (B) placenta and (C) fetal intestine, using gradient elution condition I (elution gradient shown by the dotted lines). The elution positions of human PYY₁₋₃₆, NPY and PP are indicated by the arrows in (A), (B) and (C). (D) Gel filtration profile of PYY-LI in human amnion extracts. Gel filtration chromatography was performed on Sephadex G-50 columns (1 × 120 cm), eluted with a 1 M acetic acid/0.15 M sodium chloride solution. The elution positions of Dextran Blue (V_0), NaI-¹²⁵ (V_i) and synthetic human PYY₁₋₃₆ are shown in (D). The bars represent PYY-LI concentrations (pM) in each panel.

heterogenous profiles as minor amounts of earlier eluting peaks of unknown identity were present.

Anatomical locations

Figure 3 portrays the immunohistochemical distribution of PYY-LI in the human intrauterine tissues of pregnancy. At the earliest gestation time studied (9.5 weeks), intense cytoplasmic staining for PYY-LI was present in the syncytiotrophoblast and cytotrophoblasts of the chorionic villi (Fig. 3A). There was also intense positive cytoplasmic staining for PYY-LI in the invasive (intermediate) trophoblasts of the decidual septa at 9.5 weeks. At term (39 weeks), positive staining for PYY-LI of the syncytiotrophoblast and invasive intermediate trophoblasts of the decidual septa remained present (Fig. 3B). Term fetal membranes contained positive staining for PYY-LI in amniotic epithelial cells and in chorionic trophoblasts (Fig. 3C). Positive staining was also seen in maternal decidual cells but the precise identity of these positively stained cells has not yet been established (Fig. 3C). PYY-LI staining patterns in second and third trimester tissues were identical to those seen at 9.5 weeks and at term. Preabsorption of the PYY antiserum with PYY either abolished or greatly diminished the positive staining for PYY (Fig. 3D,E,F).

Pre-incubation of the antiserum with NPY did not affect staining for PYY and non-immune rabbit serum did not produce positive staining (data not shown).

Discussion

This is the first study to demonstrate the presence of PYY₁₋₃₆ and PYY₃₋₃₆ in human placenta and fetal membranes; PYY-LI was found to be present in these intrauterine tissues as early as 9.5 weeks of gestation and remained present throughout pregnancy to term. Previous studies had reported the presence of NPY in human placenta (Petraglia *et al.* 1989, 1993) but did not report investigations into the presence of other PP family members.

Gel filtration chromatography and low resolution reverse-phase HPLC produced concordant results demonstrating a single peak of PYY-LI occurring at the elution position of PYY₁₋₃₆ in term products of human conception and human second trimester fetal intestinal tissue extracts. Using higher resolution reverse-phase HPLC conditions, this PYY-LI peak resolved into two distinct peaks co-eluting with PYY₁₋₃₆ and PYY₃₋₃₆. Similar to previous findings in human blood (Grandt *et al.* 1994) and canine

Figure 2 Reverse-phase HPLC profiles of PYY-LI present in tissue extracts of (A) amnion, (B) placenta and (C) fetal intestine, using gradient elution condition II (elution gradient shown by the dotted lines). The elution positions of human PYY₁₋₃₆ and PYY₃₋₃₆ are indicated by arrows. The bars represent PYY-LI concentrations (pM) in each panel.

adult intestine (Grandt *et al.* 1992), term products of human conception and the human fetal intestinal extracts contain approximately equal amounts of these molecular forms.

The PYY₃₋₃₆ molecular form probably arises from post-translation processing of PYY₁₋₃₆ by the high specific activity enzyme, human dipeptidyl peptidase IV (Mentlein *et al.* 1993). Similarly, endogenous NPY₃₋₃₆ has been isolated recently from porcine brain tissue extracts (Grandt *et al.* 1996). Of note, human neonates and infants have elevated cord and peripheral blood levels of PYY-LI and, on gel permeation chromatography, the majority of this plasma PYY-LI appears to be of a similar size as PYY₁₋₃₆ (Adrian *et al.* 1986). Although the physiological significance of the presence of either PYY₁₋₃₆ or PYY₃₋₃₆ in human products of conception is unknown, these results suggest that, as is the case with the enteric nervous system, PYY has been an under-investigated peptide relative to its better known family member, NPY. PYY-LI is expressed early in pregnancy and remains present to term in the cytotrophoblasts, syncytiotrophoblast, invasive intermediate trophoblasts and in amniotic epithelial cells. These cell types produce a number of biologically active agents and conceivably either or both PYY₁₋₃₆ and PYY₃₋₃₆ may function in a paracrine or endocrine fashion to regulate the release of such agents.

At least five receptor subtypes interacting with PP peptide family members have been either cloned or

identified by pharmacological studies, the Y1 and Y2 subtypes possibly being of most relevance to this study. The Y1 receptor (Herzog *et al.* 1992, Krause *et al.* 1992, Larhammar *et al.* 1992) requires an intact N-terminal region of NPY or PYY for high affinity binding. It binds NPY, PYY and (Leu³¹, Pro³⁴)-NPY with equal potency but has much lower affinity for PP and C-terminal fragments of NPY or PYY (including PYY₃₋₃₆). The Y2 receptor subtype (Gerald *et al.* 1995, Rose *et al.* 1995, Gehlert *et al.* 1996) binds NPY, PYY and C-terminal fragments of both molecules with equal potency; much lower affinity binding is seen using either PP or (Leu³¹, Pro³⁴)-NPY as ligands.

The Y1 receptor subtype is expressed in human placenta (Wharton *et al.* 1993), selectively localized to the syncytiotrophoblast, with other regions containing considerably lower levels of the message coding for this receptor subtype. As NPY has been demonstrated to result in the release of corticotrophin releasing factor from cultured human placenta cells (Petraglia *et al.* 1989, 1993), it will be of interest to determine if PYY₁₋₃₆ also has this action and if the action is mediated by the Y1 receptor subtype. The function of the PYY₃₋₃₆ molecular form is at present also unknown. PYY₃₋₃₆ would not be expected to interact with the Y1 receptor with high affinity and the presence of the Y2 receptor subtype in pregnancy-associated tissues has not been reported, as yet. Conceivably, the production of PYY₃₋₃₆ from PYY₁₋₃₆

Figure 3 Immunohistochemical staining (left panels) of PYY-LI in human intrauterine tissues: (A) placental villi (9.5 weeks), (B) placenta at term (39 weeks) and (C) fetal membranes at term (39 weeks). The panels on the right (D, E, F) show corresponding preabsorption controls. ivs, intervillous space; s, syncytiotrophoblast; c (in (A)), cytotrophoblasts; int, intermediate (extravillous) cytotrophoblasts; a, amnion; c (in (C)), chorion; d, decidua. The bar represents 60 µm.

may act as a regulatory mechanism to control the amount of available PYY₁₋₃₆ interacting with the Y1 receptor. Alternatively, PYY₃₋₃₆ could exert a unique activity via interaction with the Y2 receptor subtype, if such is shown to be present in these tissues by future studies.

Although the trophic effects of PYY or NPY have not been extensively investigated, there are reports suggesting that these peptides do have effects on growth. PYY administration is reported to be trophic to the duodenum

of the nursing but not the adult rat, and to increase significantly the weight and DNA content of the duodenum, ileum and colon of adult mice (Guan *et al.* 1993, Gomez *et al.* 1995). Interestingly, PYY inhibits cholecystokinin-stimulated pancreatic growth in adult rat and inhibits the growth of human pancreatic cancer cell lines via interaction with the Y2 receptor subtype (Liu *et al.* 1995a,b). NPY produces mitogenic effects on human and rat vascular smooth muscle cells (Zukowska-Grojec

et al. 1993, Erlinge *et al.* 1994) and on adult rat ventricular myocytes (Millar *et al.* 1994); the mitogenic effects in these reports were mediated by the Y1 receptor subtype. The presence of PYY₁₋₃₆ and the Y1 receptor subtype in human products of conception is consistent with a potential trophic role for PYY₁₋₃₆ in placental and possibly fetal development. Potential roles for PYY₃₋₃₆ in trophism would require the presence of a receptor sub-type capable of recognizing PYY₃₋₃₆ with high affinity.

In summary, we have demonstrated the presence of PYY₁₋₃₆ and PYY₃₋₃₆ in human maternal and fetal placental membranes. PYY-LI appears early in pregnancy and remains present to term. Functional studies on PYY and its interactions with specific receptor subtypes during pregnancy are required.

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References

- Adrian TE, Smith HA, Calvert SA, Aynsley-Green A & Bloom SR 1986 Elevated plasma peptide YY in human neonates and infants. *Pediatric Research* **20** 1225–1227.
- Erlinge D, Brunkwall J & Edvinsson L 1994 Neuropeptide Y stimulates proliferation of human vascular smooth muscle cells: cooperation with noradrenaline and ATP. *Regulatory Peptides* **50** 259–265.
- Fraser M, Carter AM, Challis JRG & McDonald TJ 1992 Gastrin releasing peptide immunoreactivity is present in ovine amniotic fluid and fetal and maternal circulations. *Endocrinology* **131** 2033–2035.
- Fraser M, McDonald TJ, Spindel ER, Fahy M, Hill D & Challis JRG 1994 Gastrin releasing peptide is produced in the pregnant ovine uterus. *Endocrinology* **135** 2440–2445.
- Furness JB, Costa M, Emson PC, Håkanson R, Moghizadeh E, Sundler F, Taylor IL & Chance RE 1983 Distribution pathways and reactions to drug treatment of nerves with neuropeptide Y- and pancreatic polypeptide-like immunoreactivity in the guinea-pig digestive tract. *Cell and Tissue Research* **234** 71–92.
- Gehlert DR, Beavers LS, Johnson D, Gackenhaimer SL, Schober DA & Gadsdill RA 1996 Expression cloning of a human brain neuropeptide Y Y2 receptor. *Molecular Pharmacology* **49** 224–228.
- Gerald C, Walker MW, Vaysse PJ-J, He C, Branchek TA & Weinshank RL 1995 Expression cloning and pharmacological characterization of a human hippocampal neuropeptide Y/peptide YY Y2 receptor subtype. *Journal of Biological Chemistry* **270** 26758–26761.
- Gomez G, Zhang T, Rajaraman S, Thakore KN, Yanaihara N, Townsend CM Jr, Thompson JC & Greeley GH 1995 Intestinal peptide YY: ontogeny of gene expression in rat bowel and trophic actions on rat and mouse bowel. *American Journal of Physiology* **268** G71–G81.
- Grandt D, Teyssen S, Schimiczek M, Reeve JR Jr, Feth F, Rascher W, Hirche H, Singer MV, Layer P, Goebell H, Ho FJ & Eysselein VE 1992 Novel generation of hormone receptor specificity by amino terminal processing of peptide YY. *Biochemical and Biophysical Research Communications* **186** 1299–1306.
- Grandt D, Schimiczek M, Beglinger Ch, 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.
- Grandt D, Schimiczek M, Rascher W, Feth F, Shively J, Lee TD, Davis MT, Reeve JR Jr & Michel MC 1996 Neuropeptide Y 3–36 is an endogenous ligand selective for Y2 receptors. *Regulatory Peptides* **67** 33–37.
- Guan D, Rivard N, Morisset J & Greeley GH Jr 1993 Effects of peptide YY on the growth of the pancreas and intestine. *Endocrinology* **132** 219–223.
- Herzog H, Hort YJ, Ball HJ, Hayes G, Shine J & Selbie LA 1992 Cloned human neuropeptide Y receptor couples to two different second messenger systems. *Proceedings of the National Academy of Sciences of the USA* **89** 5794–5798.
- Kimmel JR, Hayden LJ & Pollock HG 1975 Isolation and characterization of a new pancreatic polypeptide hormone. *Journal of Biological Chemistry* **250** 9369–9376.
- Krause J, Eva C, Seeburg PH & Sprengel R 1992 Neuropeptide subtype pharmacology of a recombinantly expressed neuropeptide receptor. *Molecular Pharmacology* **41** 817–821.
- Larhammar D, Blomquist AG, Yee F, Jazin E, Yoo H & Wahlestedt C 1992 Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y1 type. *Journal of Biological Chemistry* **267** 10935–10938.
- Liu CD, Balasubramaniam A, Saxton RE, Paiva M & McFadden DW 1995a Human pancreatic cancer growth is inhibited by peptide YY and BIM-43004-1. *Journal of Surgical Research* **58** 707–712.
- Liu CD, Slice LW, Balasubramaniam A, Walsh JH, Newton TR, Saxton RE & McFadden DW 1995b Y2 receptors decrease human pancreatic cancer growth and intracellular cyclic adenosine monophosphate levels. *Surgery* **118** 229–236.
- Lundberg JM, Tatemoto K, Terenius L, Hellstrom PM, Mutt V, Hökfelt T & Hamberger B 1982a Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. *Proceedings of the National Academy of Sciences of the USA* **79** 4471–4475.
- Lundberg JM, Terenius L, Hökfelt T, Martling CR, Tatemoto K, Mutt V, Polak J, Bloom SR & Goldstein M 1982b Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiologica Scandinavica* **116** 447–480.
- McDonald TJ, Wang YF, Mao YK, Broad RM, Cook MA & Daniel EE 1993 PYY: a neuropeptide in the canine enteric nervous system. *Regulatory Peptides* **44** 33–48.
- Mentlein R, Dahms P, Grandt D & Kruger R 1993 Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regulatory Peptides* **49** 133–144.
- Millar BC, Schluter K-D, Zhou X-J, McDermott BJ & Piper HM 1994 Neuropeptide Y stimulates hypertrophy of adult ventricular cardiomyocytes. *American Journal of Physiology* **266** C1271–C1277.
- Petraglia F, Calza L, Giardino L, Sutton S, Marrama P, Rivier J, Genazzani AR & Vale W 1989 Identification of immunoreactive neuropeptide-Y in human placenta: localization, secretion, and binding sites. *Endocrinology* **124** 2016–2022.
- Petraglia F, Calza L, Giardino L, Zanni M, Florio P, Ferrari AR, Nappi C & Genazzani AR 1993 Maternal decidua and fetal membranes contain immunoreactive neuropeptide Y. *Journal of Endocrinological Investigation* **16** 201–205.

- Rose PM, Fernandes P, Lynch JS, Frazier ST, Fisher SM, Kodukula K, Kienzie B & Seethala R 1995 Cloning and expression of a cDNA encoding a human type 2 neuropeptide Y receptor. *Journal of Biological Chemistry* **270** 22661–22664.
- Sangha RK, Walton JC, Ensor CM, Tai H-H & Challis JRG 1994 Immunohistochemical localization, messenger ribonucleic acid abundance, and activity of 15-hydroxy-prostaglandin dehydrogenase in placenta and fetal membranes during term and preterm labour. *Journal of Clinical Endocrinology and Metabolism* **78** 982–989.
- Schwartz TW 1983 Pancreatic polypeptide: a hormone under vagal control. *Gastroenterology* **85** 1411–1425.
- Tatemoto K 1982a Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. *Proceedings of the National Academy of Sciences of the USA* **79** 2514–2518.
- Tatemoto K 1982b Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proceedings of the National Academy of Sciences of the USA* **79** 5485–5489.
- Taylor IL 1989 The ileo-colonic, gastric and pancreatic inhibitor. *Biological Bulletin* **177** 187–191.
- Taylor IL, Impicciatore M, Carter DC & Walsh JH 1978 Effect of atropine and vagotomy on pancreatic polypeptide response to a mixed meal in dogs. *American Journal of Physiology* **235** E443–E447.
- Wang YF, Mao YK, Xiao Q, Daniel EE, Borkowski KR & McDonald TJ 1997 The distribution of NPY-containing nerves and the catecholamine contents of canine enteric nerve plexuses. *Peptides* **18** 221–234.
- Wharton J, Gordon L, Byrne J, Herzog H, Selbie LA, Moore K, Sullivan MHF, Elder MG, Moscoso G, Taylor KM, Shine J & Polak JM 1993 Expression of the human neuropeptide tyrosine receptor. *Proceedings of the National Academy of Sciences of the USA* **90** 687–691.
- Xiao Q, Challis JRG, Fraser M, Wlodek ME, Thorburn GD, Cuttita F, Hill DJ, St-Pierre S, Spindel ER & McDonald TJ 1996a Locations and molecular forms of gastrin releasing peptide-like immunoreactive entities in ovine pregnancy. *Peptides* **17** 489–495.
- Xiao Q, Han X, Challis JRG, Hill DJ, Spindel ER, Prasad CJ, Akagi K & McDonald TJ 1996b Gastrin releasing peptide-like immunoreactivity is present in human maternal and fetal placental membranes. *Journal of Clinical Endocrinology and Metabolism* **81** 3766–3773.
- Zukowska-Grojec Z, Pruszczyk P, Colton C, Yao J, Shen GH, Myers AK & Wahlestedt C 1993 Mitogenic effect of neuropeptide Y in rat vascular smooth muscle cells. *Peptides* **14** 263–268.

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