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Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV

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Summary

Neuropeptide Y, peptide YY and pancreatic polypeptide share an evolutionary conserved proline-rich N-terminal sequence, a structure generally known to be inert to the attack of common proteinases, but a potential target for specialized proline-specific aminopeptidases. Purified human dipeptidyl peptidase IV (also termed CD 26) liberated N-terminal Tyr-Pro from both, neuropeptide Y and peptide YY, with very high specific activities and K_m values in the micromolar range, but almost no Ala-Pro from pancreatic polypeptide. Other proline-specific aminopeptidases exhibited low (aminopeptidase P, liberation of N-terminal Tyr) or totally no activity (dipeptidyl peptidase II), as was also observed with less-specific aminopeptidases (aminopeptidase M, leucine aminopeptidase). When human serum was incubated with neuropeptide Y or peptide YY at micro- and nanomolar concentrations, Tyr-Pro was detected as a metabolite of both peptides. Formation of Tyr-Pro in serum was blocked in the presence of Lys-pyrrolidide and diprotin A (Ile-Pro-Ile), specific, competitive inhibitors of dipeptidyl peptidase IV. Incubation of neuropeptide Y or peptide YY with immunocytochemically defined, cultivated endothelial cells from human umbilical cord also yielded Tyr-Pro. Dipeptidyl peptidase IV could be immunostained on most endothelial cells by a specific antibody. We suggest that dipeptidyl peptidase IV might be involved in the degradation of neuropeptide Y and peptide YY to N-terminal truncated neuropeptide Y(3–36) and peptide YY(3–36). Since specific binding to Y_1, but not to Y_2 subtype of neuropeptide Y/peptide YY receptors requires intact N- as well as C-termini of neuropeptide Y and peptide YY, removal of their amino-terminal dipeptides by dipeptidyl peptidase IV inactivates them for binding to one receptor subtype.

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Abbreviations: NPY, neuropeptide Y; PYY, peptide YY; PP, pancreatic polypeptide; DPP IV, dipeptidyl peptidase IV (EC 3.4.14.5; CD 26); APP, aminopeptidase P (EC 3.4.11.9).

Introduction

Neuropeptide Y (NPY), peptide YY (PYY), and pancreatic polypeptide (PP) are 36-amino acid regulatory peptides with high homology in primary (Fig. 1) as well as tertiary structure. NPY is widely distributed in central and peripheral neurons, PYY is produced by endocrine cells of the small intestine and colon, and PP occurs in distinct cells of the islets of Langerhans. NPY and PYY appear to bind to common receptors with equal affinity, whereas receptors for PP are different [1]. Furthermore, at least three subtypes of NPY and PYY receptors, Y_1, Y_2 and Y_3, exist [2,3].

In the primary structure of these three peptides the proline-residues at positions 2, 5 and 8 are highly conserved among species (compare Ref. 4) as well as among the different members of the peptide family (Fig. 1). Since sequences involving proline bonds are resistant to common proteinases, but on the other hand are targets for specialized enzymes [5], we evaluated whether or not proline-specific proteinases might metabolize these peptides to inactive fragments or those with modified receptor specificity. In this report we present evidence that NPY(3–36) and PYY(3–36) are generated from the parent peptides by action of dipeptidyl peptidase IV (DPP IV), a highly specialized aminopeptidase removing only

1 5 10 15 36

Tyr-**Pro**-Ser-Lys-**Pro**-Asp-Asn-**Pro**-Gly-Glu-Asp-Ala-**Pro**-Ala-Glu-...$TyrNH_2$ *human* NPY

Tyr-**Pro**- Ile-Lys-**Pro**-Glu-Ala -**Pro**-Gly-Glu-Asp-Ala-Ser-**Pro**-Glu-...$TyrNH_2$ *human* PYY

Ala-**Pro**-Leu-Glu-**Pro**-Val-Tyr-**Pro**-Gly-Asp-Asn-Ala-Thr-**Pro**-Glu-...$TyrNH_2$ *human* PP

Tyr-**Pro**-Ser-Lys-**Pro**-Asp-Asn-**Pro**-Gly-Glu-Gly-Ala-**Pro**-Ala-Glu-...$TyrNH_2$*dogfish* NPY

Tyr-**Pro**-Pro-Lys-**Pro**-Glu-Asn-**Pro**-Gly-Glu-Gly-Ala-**Pro**-Pro-Glu-...$TyrNH_2$*dogfish* PYY

Thr-**Pro**-Leu-Gln-**Pro**-Lys-Tyr-**Pro**-Gly-Asp-Gly-Ala-**Pro**-Val-Glu-...$TyrNH_2$ *alligator* PP

Fig. 1. N-terminal sequences of human neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) as compared to sequences of lower vertebrates. Proline residues (bold) in positions 2, 5 and 8 are highly conserved in all sequences of this peptide family as well as in different species (dogfish sequences taken from Ref. 4).

Xaa-Pro (and Xaa-Ala) dipeptides from N-terminal sequences with second Pro (or Ala). We suggest that this processing may regulate the biological activity of NPY and PYY by influencing their binding to different receptor subtypes, because intact N- and C-terminal sequences are required for interaction with Y_1-receptors, whereas binding to the Y_2-receptor subtype is achieved also by fragments of the C-terminal parts alone [6,7].

Materials and Methods

Peptides and enzymes

Synthetic NPY and PYY were purchased from Saxon Biochemicals, Hannover, Germany, and other peptides were from Bachem, Bubendorf, Switzerland. All peptides contained the human sequences. NPY or PYY (2 nmol each) were radioiodinated by the chloramine T method and purified by reverse-phase HPLC [8]. The specific activity calculated from amount of peptide and recovered radioactivity was 10 μCi/nmol. Lys-pyrrolidide was a gift from Dr. Mike Schutkowski, Martin-Luther-Universität, Halle/Saale, Germany. DPP IV (EC 3.4.14.5) was purified from human placenta [9], dipeptidyl peptidase II (EC 3.4.14.2) and aminopeptidase P (EC 3.4.11.5) were isolated from rat brain as described [10,11]. Aminopeptidase M (EC 3.4.11.2), leucine aminopeptidase (EC 3.4.11.1) and prolidase (EC 3.4.13.9), all from hog kidney, were obtained from Sigma, Munich, Germany; they were pretreated with 1 mM diisopropylfluorophosphate to ensure absence of contaminating DPP IV activity [12].

Enzymatic assays

5 nmol of the peptides (5 μl of a 1 mM solution in water) were incubated at 37°C with 1 ng peptidase or 3 μl human serum in 50 mM triethanolamine/HCl buffer, pH 7.8, for 5–30 min in a total volume of 500 μl (final peptide concentration 10 μM). For kinetic assays peptide concentrations were varied between 50–0.5 μM. Peptides with Tyr could be quantified down to 0.1 nmol. Radioiodinated NPY or PYY (approx. 220,000 cpm corresponding to 10 pmol) were incubated in buffer supplemented with 0.05% bovine serum albumin to reduce adsorption to glass and plastic surfaces. Assays with purified aminopeptidase P were supplemented with 1 mM dithiothreitol and 1 mM $MnCl_2$. Dipeptidyl peptidase II was incubated with the peptides in 50 mM acetate buffer, pH 5.5. Enzymatic reactions were terminated by addition of 5 μl trifluoroacetic acid, and applied onto a Spherisorb ODS-2 (3 μm particles, 125 × 4 mm; Pharmacia, Freiburg, Germany) reverse-phase HPLC column. Peptides and their fragments, including liberated Tyr, were separated by a linear gradient of 0–80% acetonitrile in 0.1% trifluoroacetic acid formed within 15 min at a flow rate of 1 ml/min, and quantified by their absorbance at 220 nm in the eluate. Presence of Tyr-Pro was either verified by boiling (5 min) of incubation samples before acidification, subsequent incubation (30 min, 37°C) with 10–50 μg desalted prolidase, and processing as above. In some experiments fragments were collected after HPLC, hydrolysed with 6 M HCl in vacuo (24 h, 100°C), derivatized with 4-dimethylaminoazobenzene-4′-sulfonylchloride (dabsyl-chloride), and derivates were separated by reverse-phase HPLC [13]. Free amino acids and Ala-Pro were directly lyophilized, derivatized and analyzed as above. Dabsyl-Ala-Pro elutes between Dabsyl-Pro and Dabsyl-Val. Degradation rates were calculated from the areas of liberated dipeptides/amino acids quantified by standards.

Standard assays for DPP IV with 0.5 mM Gly-Pro-4-nitranilide and for aminopeptidase P with 1 mM Arg-Pro-Pro have been reported earlier [9–11,14]. Protein contents of purified enzymes were calculated from their activities with standard substrates [9–11,14].

Cell culture and immunocytochemistry

Endothelial cells were obtained from human umbilical veins, cultivated and subcultivated twice as described by Jaffe et al. [15] with a few modifica-

tions: cells were plated on collagen (from rat tail) coated petri dishes or coverslips (for immunocytochemistry), and incubation medium was supplemented with 50 ng/ml basic fibroblast growth factor and 50 μg/ml heparin. Prior to degradation experiments cells were washed twice carefully with prewarmed 145 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM glucose, 20 mM Hepes, pH 7.4. Washed cells were then incubated with 1 ml of 10 μM peptide in this buffer for 0–90 min at 37°C.

Immunocytochemistry was performed with cells fixed by cold acetone with either rabbit anti-human factor VIII antiserum (Dakopatts, Hamburg, Germany) or rabbit anti-human DPP IV [16] in phosphate-buffered saline (1:20, 24 h, 4°C) followed by fluoresceine isothiocyanate labelled anti-rabbit immunoglobulin G as second antibody (1:20, 30 min). After washing cultures were counterstained with bisbenzimide (0.3% in phosphate-buffered saline), washed, embedded in saline-glycerol (9:1). Green fluorescence of fluoresceine immunostaining was inspected at 510–560 nm emission and blue nuclear counterstain at 450–490 nm emission from the same sample irradiated at 390–420 nm excitation. DNA in cultivated cells was measured by the ethidium bromide method [17] after lysis in hypotonic buffer and scraping off by a rubber-policeman.

TABLE I

Cleavage of 10 μM neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP, human sequences) by highly purified dipeptidyl peptidase IV (DPP IV, from human placenta), dipeptidyl peptidase II (DPP II, from rat brain) and aminopeptidase P (APP, from rat brain)

All assays were performed under conditions optimal for the enzyme selected, see Materials and Methods. No activity was measured with the less-specific enzymes aminopeptidase M and leucine aminopeptidase.

Peptide	Cleavage rates (μmol min^{-1} mg^{-1}) by		
	DPP IV	DPP II	APP
NPY	27	<0.01	0.2
PYY	6.8	<0.01	0.2
PP	<0.01	<0.01	<0.1

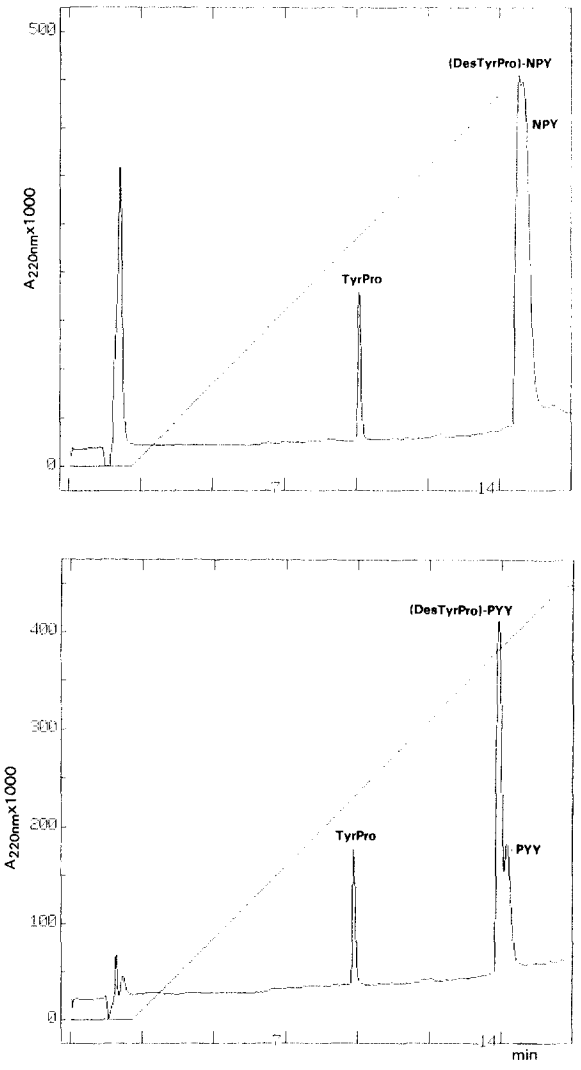

Fig. 2. Reverse-phase high pressure liquid chromatographic separations of dipeptidyl peptidase IV digests of neuropeptide Y (NPY) and peptide YY (PYY). The positions of liberated Tyr-Pro (determined with standard) and of the truncated peptides (only partly separated) are indicated. The octadecyl HPLC column was eluted with a linear gradient of 0–80% acetonitrile in 0.1% trifluoroacetic acid, and peptides were monitored in the eluate by their absorbance at 220 nm, see Materials and Methods.

Results

Degradation of NPY and PYY by purified proteinases

The dipeptidyl peptidases IV and II and aminopeptidase P are the only enzymes so far known to hydrolyse N-terminal Xaa-Pro-sequences by removal of the terminal dipeptide or amino acid [5]. NPY, PYY and PP were digested at different rates by these proteinases. NPY and PYY were cleaved with high rates by DPP IV and with lower ones by aminopeptidase P, whereas PP was not measurably attacked (Table I). Dipeptidyl peptidase II or the less-specific enzymes aminopeptidase M and leucine aminopeptidase did not cleave either NPY, PYY nor PP.

In accordance with their known specificity DPP IV liberated only N-terminal Tyr-Pro (Fig. 2) and aminopeptidase P N-terminal Tyr (not shown) from NPY and PYY even after prolonged (180 min) incubations. Combined actions of DPP IV with purified leucine aminopeptidase or aminopeptidase M (both in 100-fold surplus by weight) on NPY yielded only few, small digestion peaks beside Tyr-Pro (not shown). This indicates a relatively high stability of NPY(3–36) to further aminopeptidase attack which should subsequently liberate Ser, Lys-Pro and so on.

In a further set of experiments we evaluated whether DPP IV is also active on NPY and PYY at physiological, nanomolar concentrations. Due to sensitivity of photometric detection, determination of kinetic constants could be performed down to 500 nM with the unlabelled peptides (Table II). Degradation of NPY and PYY did not obey Michaelis-Menten kinetics at concentrations above 20 μM. The observed overproportional increase of peptidase activity above 20 μM has been observed earlier with other neuropeptides, e.g., substance P [14]. However, double reciprocal plots according to Lineweaver and Burk were linear below 20 μM and were used for the determination of apparent K_m- and V_{max}/k_{cat} values (Table II). Turnover rates are very high for NPY and for PYY, both exceeding those determined for other peptide hormone substrates, e.g., substance P. As found for other peptide-degrading enzymes [18] K_m values for NPY and PYY are in the μM-range. The actions of peptidases at lower peptide concentrations are given by the rate constants k_{cat}/K_m: high rate constants indicate high digestion rates at nanomolar concentrations. High k_{cat}/K_m values were found for DPP IV cleavage of both peptides, especially for NPY. Moreover, DPP IV released labelled Tyr-Pro from radioiodinated NPY and PYY when these labelled peptides were incubated with purified proteinase even at a nanomolar concentration (Fig. 3). This proves an action at physiological concentrations.

TABLE II

Kinetic constants for the cleavage of neuropeptide Y and peptide YY compared to other peptides by human dipeptidyl peptidase IV

β-Casomorphin and substance P are established substrates for dipeptidyl peptidase IV and data [14] are added for comparison. The k_{cat} values were calculated on the basis of 120 kDa for one active subunit of dipeptidyl peptidase IV which is dimeric in solution. Substrate activation was observed at concentrations above 20 μM for NPY and PYY, above 50 μM for substance P.

Peptide	N-terminus	Range (μM)	K_m (μM)	V_{max} (μmol min^{-1} mg^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} M^{-1})
Neuropeptide Y	YP-SKP...	50–0.5	8	48	96	$12.0 \cdot 10^6$
Peptide YY	YP-IKP...	50–0.5	28	26	52	$1.9 \cdot 10^6$
β-Casomorphin	YP-FP-GP-I	500–20	59	90	180	$3.1 \cdot 10^6$
Substance P	RP-KP-QQ...	400–25	22	10	20	$0.54 \cdot 10^6$

Degradation of NPY and PYY in serum

It is known that serum contains activity of soluble DPP IV and aminopeptidase P [13]. Therefore, we evaluated whether these proteinases might be involved in the degradation of NPY and PYY in human serum. In sera of healthy males ($n = 6$) activities of 55 ± 12 μmol min^{-1}l^{-1} for DPP IV were measured with the chromogenic substrate Gly-Pro-4-nitranilide (0.5 mM, 37°C) and of 12 ± 8 μmol min^{-1}l^{-1} for aminopeptidase P with Arg-Pro-Pro (1 mM, 37°C). The relatively high variability of serum aminopeptidase P activity may result from a different release after coagulation from platelets where this enzyme is found [19].

When 10 μM NPY was incubated with a small amount of serum with mean DPP IV and high (20 μmol min^{-1}l^{-1}) aminopeptidase P activities at 37°C, Tyr-Pro was found as a metabolite (Fig. 4). The formation of Tyr-Pro by human serum was verified by (i) identical retention time with standard, and

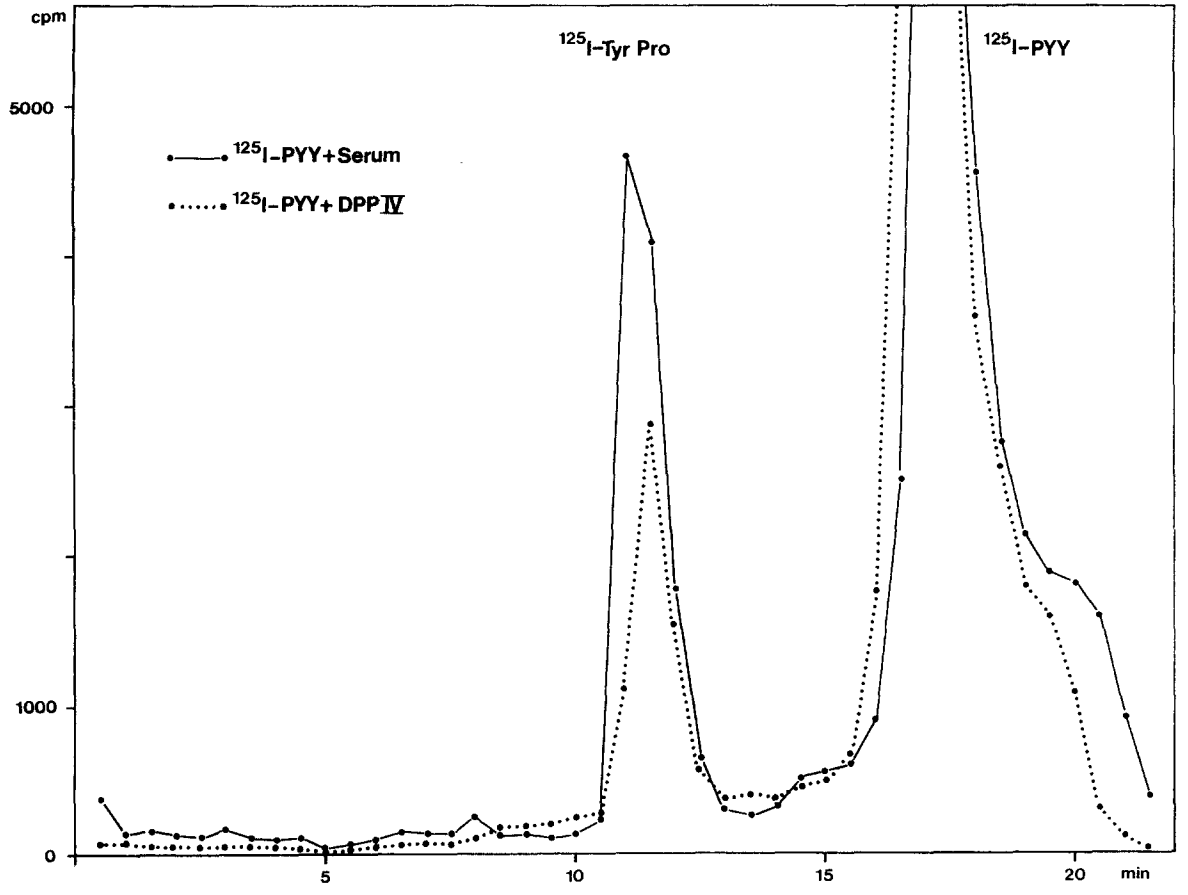

Fig. 3. Degradation of radioiodinated peptide YY (PYY) by purified dipeptidyl peptidase IV (dotted line) and by human serum (solid line). Similar results were obtained with radioiodinated neuropeptide Y (NPY). In the experiments shown, 20 nM ^{125}I-PYY was incubated with 6 ng purified dipeptidyl peptidase IV or 100 μl serum in 500 μl triethanolamine buffer, pH 7.4, containing 0.05% bovine serum albumin for 60 min at 37°C. Acidified samples were analyzed by HPLC as described in Fig. 2, fractions of 0.5 ml collected and measured for radioactivity. Note that ^{125}I-Tyr-Pro is more lipophilic than the unlabelled dipeptide and therefore does not elute at the same position! Examples of representative chromatograms where nearly all ^{125}I-Tyr-Pro is liberated from ^{125}I-PYY applied.

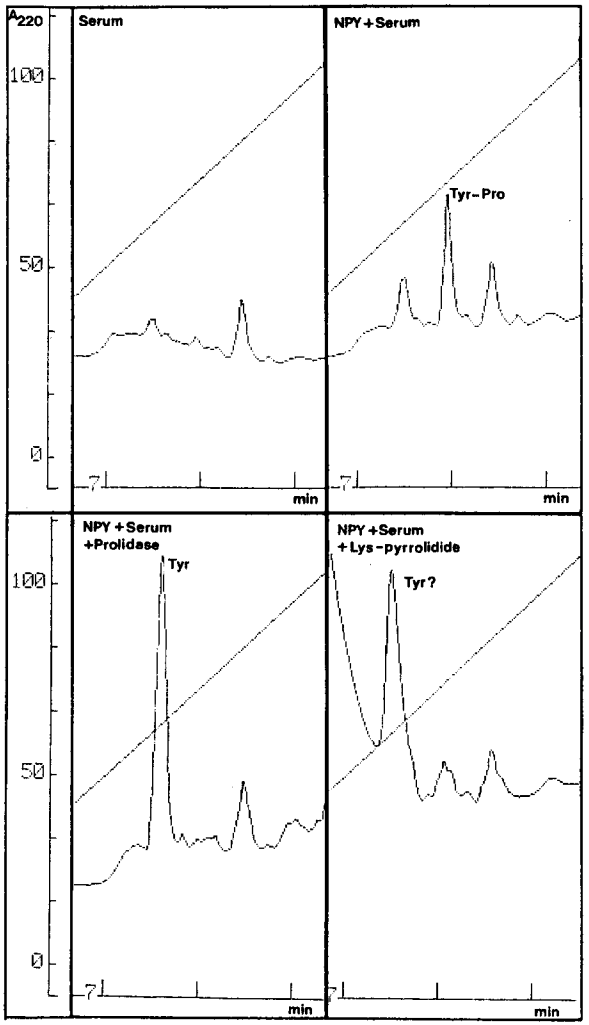

Fig. 4. Degradation of neuropeptide Y (NPY) by human serum, identification of fragments and influence of the selective, competitive dipeptidyl peptidase IV inhibitor Lys-pyrrolidide. 3 μl serum were incubated with 10 μM NPY in 500 μl triethanolamine buffer, pH 7.8, for 60 min at 37°C, acidified and analyzed by HPLC as described in Materials and Methods. Pictures show parts of HPLC runs as in Fig. 2 depicted from incubations as indicated of: (a) serum blank, (b) NPY and serum, (c) NPY and serum, stopped and further digested by prolidase which splits Tyr-Pro to Tyr and Pro (no signal at 220 nm), and (d) NPY and serum in the presence of the selective dipeptidyl peptidase inhibitor Lys-pyrrolidide (1 mM). A peak at the position of Tyr is observed (impurity of Lys-pyrrolidide or Tyr as fragment?).

(ii) disappearance of the Tyr-Pro-peak and appearance of Tyr (retention time as a standard) after further digestion of the heat-inactivated incubation sample with purified prolidase, a strict dipeptidase known to act only on Xaa-Pro dipeptides (Fig. 4), and (iii) determination of the amino acid composition of this fragment collected after HPLC separation.

Moreover, almost no Tyr-Pro was detected when serum was incubated with NPY in the presence of specific inhibitors of DPP IV, 1 mM lysylpyrrolidide (Fig. 4) or 0.1 mM diprotin A. In contrast, incorporation of 0.1 mM bestatin, an inhibitor of common aminopeptidases, had no effect on Tyr-Pro formation (not shown). Lys-pyrrolidide (Lys-tetrahydropyrrole) is a substrate analog and acts as a competitive inhibitor of dipeptidyl peptidases IV and II, but not of aminopeptidase P or other proteinases (as far as tested by us), and inhibited at 1 mM concentration purified or serum DPP IV activity (measured with 0.5 mM Gly-Pro-4-nitranilide) completely. Diprotin A (Ile-Pro-Ile) is a bad, but high affinity substrate ($K_m = 4\ \mu M$) for DPP IV [20] and reduced this activity in serum measured with the chromogenic substrate at 0.1 mM to 9%.

Furthermore, incubation of radioiodinated NPY or PYY at nanomolar concentration with serum yielded a radiolabelled digestion product eluting at the same position as radioiodinated Tyr-Pro obtained by digestion of labelled NPY or PYY with purified DPP IV (Fig. 3). NPY and PYY contain both 5 Tyr to be radioiodinated, of which Tyr-1 and Tyr-36 are preferentially labelled [21]. Thus, Tyr-Pro and the truncated peptides or further metabolites of the C-terminal parts of the peptides can be detected. As concluded from these digestion patterns (Figs. 3 and 4), DPP IV appears to be involved in the catabolism of NPY and PYY in human serum.

Degradation of NPY by cultivated endothelial cells

Blood vessels have been reported as a major tissue localisation for DPP IV [22]. We therefore incubated cultivated endothelial cells with NPY and

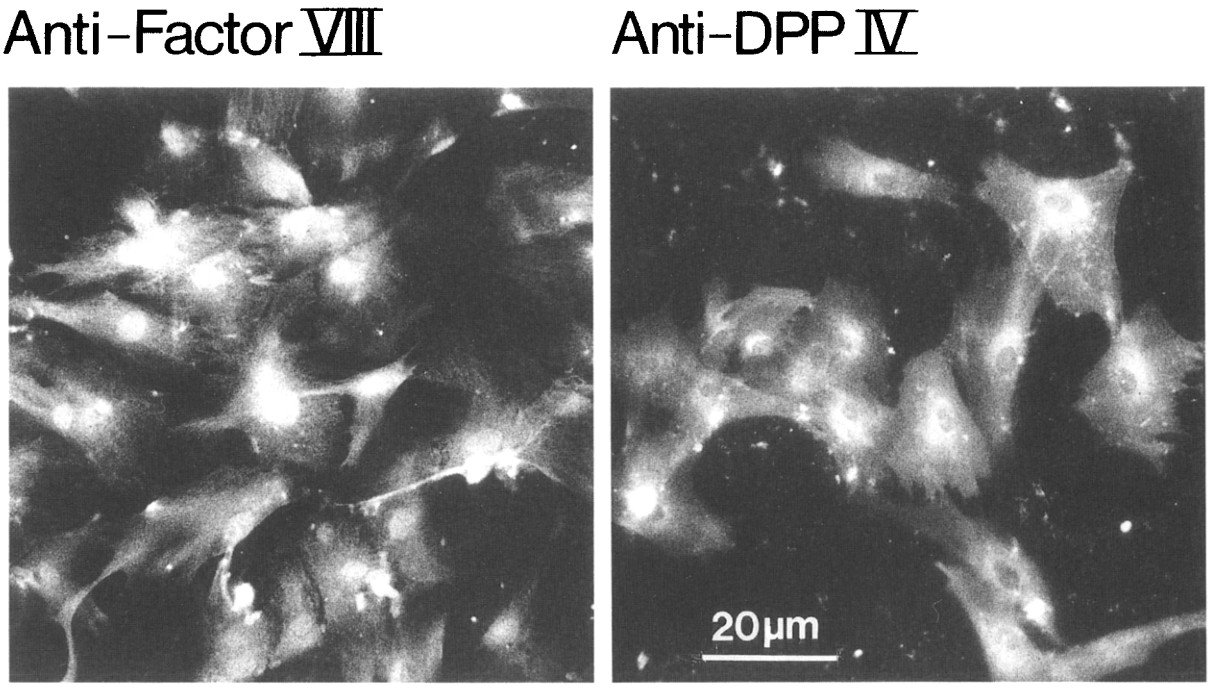

Fig. 5. Immunostaining of cultivated endothelial cells for factor VIII (anti-Factor VIII), an established endothelial marker, and for dipeptidyl peptidase IV (anti-DPP IV). Samples were incubated with the specific primary, polyclonal rabbit antisera, and subsequently with fluoresceine-conjugated anti-rabbit immunoglobulin G as second antibody. For checking the purity of cultures, nuclear counterstaining performed with bisbenzimide (not shown), and fluorescence of immune (green) and nuclear (blue) stainings were inspected together. Nearly all cultivated cells were positive for factor VIII and for dipeptidyl peptidase IV.

PYY. Endothelial cells were obtained from human umbilical cord, and after two subcultures more than 90% of the cells were positive for factor VIII, an established endothelial cell marker (Fig. 5). In homogenates of these cells, specific activities of 1.6 $nmol\,min^{-1}\,\mu g\ DNA^{-1}$ were measured for the hydrolysis of Gly-Pro-4-nitranilide as standard DPP IV chromogenic substrate, but aminopeptidase P activity was not detectable with Arg-Pro-Pro (= bradykinin(1–3)).

Again, after incubation of cultivated endothelial cells with NPY or PYY, Tyr-Pro was detected as a metabolite in cell supernatants (Fig. 6). As in corresponding assays with serum, identity of this main digestion peak with Tyr-Pro was verified by (i) identical retention time with a standard, and (ii) disappearance of Tyr-Pro and appearance of Tyr after further digestion of the heat inactivated sample with prolidase (Fig. 6). Despite other peaks in the HPLC runs, it is evident that cultivated endothelial cells liberate of N-terminal Tyr-Pro from NPY and PYY.

Discussion

NPY and PYY, less PP, are cleaved with high specific activities and high rate constants to their des(Tyr-Pro) fragments by DPP IV. These kinetic constants exceed those determined earlier for the digestion of other bioactive peptides with terminal Xaa-Pro by DPP IV, e.g., substance P, gastrin-releasing peptide or prolactin (Table II [14]). K_m

values in the micromolar range have been reported for all other neuropeptide/peptide hormone-degrading proteinases, e.g., Leu-enkephalin is hydrolyzed by 'enkephalinase' (= endopeptidase-24.11) with $K_m = 86\ \mu M$ and $k_{cat}/K_m = 0.73 \cdot 10^6 s^{-1} M^{-1}$ or angiotensin I by angiotensin-converting enzyme (= peptidyl dipeptidase A) with $K_m = 4\ \mu M$ and $k_{cat}/K_m = 2.1 \cdot 10^6 s^{-1} M^{-1}$ [18]. These (and other [18]) data are in these same range as found for NPY and PYY with DPP IV (compare Table II). Because at physiological, nanomolar peptide concentrations reaction rates v of peptide hormone-/neuropetide-degrading enzymes E are given by $v = [E] \cdot [S] \cdot k_{cat}/K_m$ [23], the value of the specifity constant k_{cat}/K_m compares best their action on competing peptide substrates. Furthermore, since the enzymes act in linear ranges of V versus [S] plots at low concentrations, their digestion rates increase with rising peptide concentrations and are not saturated (in contrast to receptors).

Though it is known that chromogenic dipeptidyl substrates or peptides with terminal aromatic or lipophilic residues (P_2-position) are good substrates for DPP IV [13,14], the sequence subsequent to the cleavage site appears to determine significantly their digestion rates. Cleavage of PP – if any – is negligible and contrasts the very high digestion rates for NPY and PYY. This might be explained by different conformational structures of NPY/PYY and PP in solution: the N-terminal segment of NPY has been found disordered in solution, whereas it folds in a polyproline-like helix in the case of PP [24]. It should

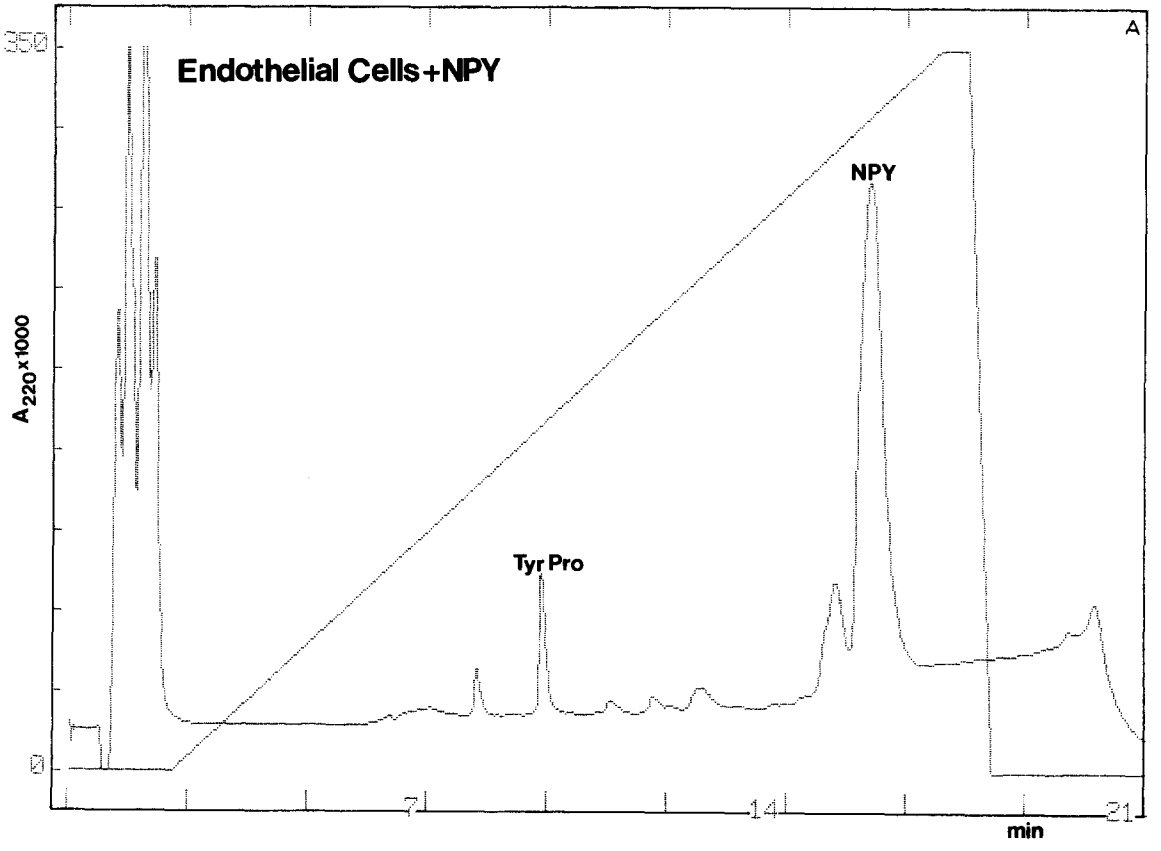

Fig. 6A. For legend see page 142.

be noted also that NPY/PYY-receptors are different from PP-receptors [1].

DPP IV has been detected in high amounts at the surface of capillary endothelial cells in situ and on surrounding muscle cells [22], in serum [13], on a subset of T-lymphocytes ([16], as leucocyte differentiation marker also termed CD 26), on hepatocytes (here also termed GP110 or OX-61 antigen [25]) as well as at intestinal and kidney brush border membranes [18]. Therefore, DPP IV should act at least on circulating PYY which is released postprandially from endocrine cells of the gut into the blood stream.

We evaluated whether the conclusions from kinetic data of the purified enzyme and its established localization might be of biological importance by two in vitro assays. When human serum and endothelial cells were incubated with NPY and PYY the characteristic, peptidase-derived dipeptide Tyr-Pro was obtained. Moreover, specific inhibitors of DPP IV abolished its formation of by serum.

The relevance of DPP IV cleavage for the degradation of NPY and PYY is further sustained by earlier reports about the occurrence of corresponding NPY and PYY fragments in tissue extracts: PYY(3–36) has been isolated from human intestine and is present in serum up to 30–60% of the total PYY-

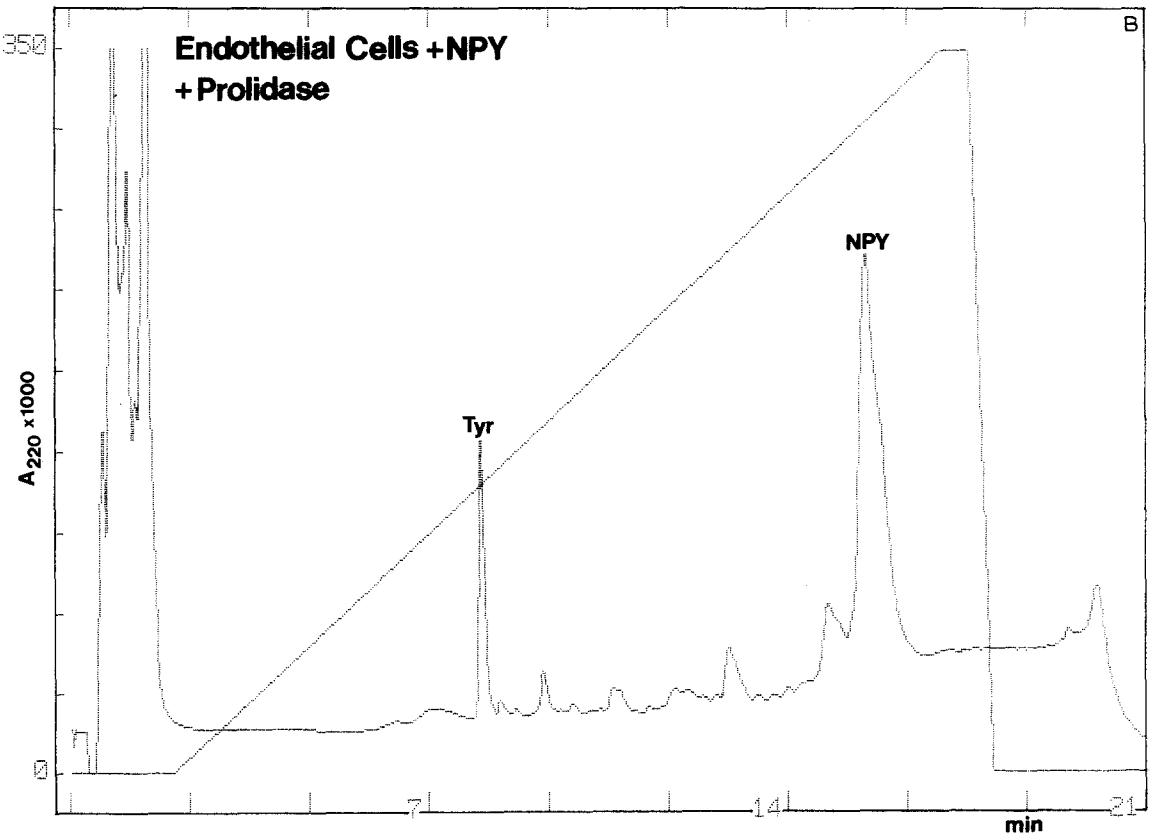

Fig. 6. Degradation of neuropeptide Y (NPY) by cultivated endothelial cells (Endothelial Cells + NPY) (A), and identification of the fragment Tyr-Pro by subsequent digestion with prolidase (Endothelial Cells + NPY + Prolidase) (B) to Tyr and Pro (not visible at 220 nm). Washed cells (corresponding to 20 μg DNA) were incubated in serum-free buffer with 10 μM NPY for 60 min at 37°C, and cell supernatants were analyzed by HPLC as in Fig. 2.

immunoreactivity [26]. NPY(3–36) has been purified from porcine brain [27]. We speculate therefore that NPY and PYY are cleaved by DPP IV in vivo to their des(Tyr-Pro)-truncated forms.

For the synthetic, N-terminal truncated peptides NPY(3–36), PYY(3–36) and to a lesser extent even NPY(2–36) it has been shown that they lack full binding and biological activity to interact with the Y_1-, but not with the Y_2-receptor subtype [7,28]. Therefore, the proteolytic cleavage of NPY and PYY by DPP IV (and even aminopeptidase P, if high concentrations are present) results in inactivation of these peptides with respect to biological effects mediated by one receptor subtype, but not by the other. Since the complete peptides affect both receptor subtypes, but the DPP IV-cleaved ones only the Y_2-receptor, biphasic responses or a certain compartmentation of the biological effects are possible. This could affect Y_1 specific peripheral bioactivities like vasoconstriction, bronchial smooth muscle cell contraction and certain gastrointestinal effects.

As examples, PYY(3–36) has been reported to be significantly less potent than intact PYY to inhibit pentagastrin-stimulated gastric acid secretion as well as deoxy-D-glucose-stimulated insulin release, but not pancreatic exocrine secretion [29]. NPY is colocalized in sympathetic perivascular nerve fibres with noradrenaline [28]. It is a vasoconstrictor alone and potentiates the action of noradrenaline on vascular smooth muscle contraction [28]. Vasoconstrictor effects are predominately mediated by Y_1 receptors [2,28] which might be affected by DPP IV.

Proteolytic cleavage is an important step for the generation of active peptide hormones from their precursors as well as for the inactivation of circulating peptides. Alteration of receptor specificity by proteolytic digestion is known for few examples and a novel biological function of dipeptidyl peptidases.

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References

1 Schwartz, T.W., Sheikh, S.P. and O'Hare, M.M.T., Receptors on phaeochromocytoma cells for two members of the PP-fold family – NPY and PP, FEBS Lett., 225 (1987) 209–214.
2 Wahlestedt, C., Grundemar, L., Håkanson, R., Heilig, M., Shen, G.H., Zukowska-Grojec, Z. and Reis, D.J., Neuropeptide Y receptor subtypes, Y1 and Y2, Ann. N.Y. Acad. Sci., 611 (1990) 7–26.
3 Wahlestedt, C., Regunathan, S. and Reis, D.J., Identification of cultured cells selectively expressing Y1-, Y2-, or Y3-type receptors for neuropeptide Y/peptide YY, Life Sci., 50 (1992) PL7–PL12.
4 Conlon, J.M., Bjenning, C. and Hazon, N., Structural characterization of neuropeptide Y from the brain of the dogfish, *Scyliorhinus canicula*, Peptides, 13 (1992) 493–497.
5 Mentlein, R., Proline residues in the maturation and degradation of peptide hormones and neuropeptides, FEBS Lett., 234 (1988) 251–256.
6 Boublik, J.H., Spicer, M.A., Scott, N.A., Brown, M.R. and Rivier, J.E., Biologically active neuropeptide Y analogs, Ann. N.Y. Acad. Sci., 611 (1990) 27–34.
7 Schwartz, T.W., Fuhlendorf, J., Kjems, L.L., Kristensen, M.S., Vervelde, M., O'Hare, M., Krstenansky, J.L. and Bjornholm, B., Signal epitopes in the three-dimensional structure of neuropeptide Y, interaction with Y_1, Y_2 and pancratic receptors, Ann. N.Y. Acad. Sci., 611 (1990) 35–47.
8 Mentlein, R., Buchholz, C. and Krisch, B., Somatostatin-binding sites on rat telencephalic astrocytes. Light-and electron-microscopic studies in vitro and in vivo, Cell Tissue Res., 258 (1990) 309–317.
9 Püschel, G., Mentlein, R. and Heymann, E., Isolation and characterization of dipeptidyl peptidase IV from human placenta, Eur. J. Biochem., 126 (1982) 359–365.
10 Mentlein, R. and Struckhoff, G., Purification of two dipeptidyl aminopeptidases II from rat brain and their action on proline-containing neuropeptides, J. Neurochem., 52 (1989) 1284–1293.
11 Harbeck, H.-T. and Mentlein, R., Aminopeptidase P from rat brain, purification and action on bioactive peptides, Eur. J. Biochem., 198 (1991) 451–458.
12 Heymann, E. and Mentlein, R., Complementary action of dipeptidyl peptidase IV and aminopeptidase M in the digestion of β-casein, J. Dairy Res., 53 (1985) 229–236.
13 Mentlein, R., Gallwitz, B. and Schmidt W.E., Dipeptidyl pep-

tidase IV hydrolyses gastric inhibitory polypeptide (GIP), glucagon-like peptide-1(7–36)amide, peptide histidine methionine (PHM) and is responsible for their degradation in human serum, Eur. J. Biochem., 214 (1993) 829–835.

14 Nausch, I., Mentlein, R. and Heymann, E., The degradation of bioactive peptides and proteins by dipeptidyl peptidase IV from human placenta, Biol. Chem. Hoppe-Seyler, 371 (1990) 1113–1118.

15 Jaffe, E.A., Nachman, R.L., Becker, C.G. and Minick, C.R., Culture of human endothelial cells derived from umbilical veins, identification by morphologic and immunologic criteria, J. Clin. Invest., 2 (1973) 2745–2756.

16 Mentlein, R., Heymann, E., Scholz, W., Feller, A.C. and Flad, H.-D., Dipeptidyl peptidase IV as a new surface marker for a subpopulation of human T-lymphocytes, Cell. Immunol., 89 (1984) 11–19.

17 Karsten, U. and Wollenberger, A., Improvements of the ethidium bromide method for direct fluorometric estimation of DNA and RNA in cell and tissue homogenates, Anal. Biochem., 77 (1977) 464–470.

18 Kenny, A.J., Stephenson, S. and Turner, A.J., Metabolism of neuropeptides. In A.J. Kenny and A.J. Turner (Eds.), Mammalian Ectoenzymes, Elsevier, Amsterdam, 1987, pp. 169–210.

19 Scharpé, S.L., Vanhoof, G.C., De Meester, I.A., Hendriks, D.F., Van Sande, M.E., Muylle, L.M. and Yaron, A., Exopeptidases in human platelets: an indication for proteolytic modulation of biologically active peptides, Clin. Chim. Acta, 195 (1990) 125–132.

20 Rahfeld, J., Schierhorn, M., Hartrodt, B., Neubert, K. and Heins, J., Are diprotin A (Ile-Pro-Ile) and diprotin B (Val-Pro-Leu) inhibitors or substrates for dipeptidyl peptidase IV?, Biol. Chem. Hoppe-Seyler, 372 (1991) 313–318.

21 Sheikh, S.P., O'Hare, M.M.T., Tortora, O. and Schwartz, T.W., Binding of monoiodinated neuropeptide Y to hippocampal membranes and human neuroblastoma cell lines, J. Biol. Chem., 264 (1989) 6648–6654.

22 Loijda, Z., Studies on dipeptidyl(amino)peptidase IV. II. Blood vessels, Histochemistry, 59 (1979) 153–166.

23 Fersht, A., Enzyme Structure and Mechanism, 2nd Edn., pp. 105–106, p. 112, W.H. Freeman and Company, New York, 1985.

24 Cowley, D.J., Hoflack, J.M., Pelton, J.T. and Saudek, V., Structure of neuropeptide Y dimer in solution, Eur. J. Biochem., 205 (1992)1099–1106.

25 McCaughan, G.W., Wickson, J.E., Cheswick, P.F. and Gorrell, M.D., Identification of the bile canalicular cell surface molecule GP110 as the ectopeptidase dipeptidyl peptidase IV: an analysis by tissue distribution, purification and N-terminal amino acid sequence, Hepatology, 11 (1990) 534–544.

26 Eberlein, G.A., Eysselein, V.E., Schaeffer, M., Layer, P., Grandt, D., Goebell, H., Niebel, W., Davis, M., Lee, T.D., Shively, J.E. and Reeve, J.R. Jr., A new molecular form of PYY: structural characterization of human PYY(3–36) and PYY(1–36), Peptides, 10 (1989) 797–803.

27 Grandt, D., Schimiczek, M., Feth, F., Ahrens, O., Rascher, W.,, Singer, M.P., Goebell, H., Reeve, J.R. Jr. and Esselein, V.E., Discovery of a new molecular form of neuropeptide Y from porcine brain, NPY 3–36, which selectively binds to Y2 receptors, Regul. Pept., 40 (1992) 161.

28 Grundemar, L., Jonas, S.E., Morner, N. Hogestatt, E.D., Wahlestedt, C. and Håkanson, R., Characterization of vascular neuropeptide Y receptors, Br. J. Pharmacol., 105 (1992) 45–50.

29 Yoshinaga, K., Mochizuki, T., Yanaihara, N., Oshima, K., Izukura, M., Kogire, M., Sumi, S., Gomez, G., Uchida, T., Thompson, J.C. and Greeley, G.H., Structural requirements of peptide YY for biological activity at enteric sites, Am. J. Physiol., 263 (1992) G695–G701.