ELSEVIER

European Journal of Pharmacology 362 (1998) 245–249

ejp

Pharmacological profile of the rat intestinal crypt peptide YY receptor vs. the recombinant rat Y5 receptor

Mathieu Goumain [a], Thierry Voisin [a], Anne-Marie Lorinet [a], Ambikaipakan Balasubramaniam [b], Marc Laburthe [a,*]

[a] *Unité de Neuroendocrinologie et Biologie Cellulaire Digestives, INSERM U410, Faculté de Médecine Bichat, B.P. 416, 75870 Paris, Cedex 18, France*
[b] *Department of Surgery, University of Cincinnati Medical Center, Cincinnati, OH 45267, USA*

Received 12 October 1998; accepted 16 October 1998

Abstract

Peptide YY and neuropeptide Y have potent antisecretory effects in rat small intestine. Scatchard analysis of [^{125}I]peptide YY binding revealed a 10-fold higher concentration of receptors in rat jejunal crypt cells than in villus cells and no detectable receptors in colonic epithelium. Reverse transcription polymerase chain reaction analysis of neuropeptide Y Y5 receptor mRNA indicated that they are mainly expressed in rat jejunal crypts with very few or no expression in villus cells and colon epithelium, respectively. In order to determine whether neuropeptide Y Y5 receptors could represent the intestinal crypt receptor for peptide YY and neuropeptide Y, the ability of peptide YY, neuropeptide Y, pancreatic polypeptide and analogues to inhibit [^{125}I]peptide YY binding to membrane prepared from rat crypt cells and COS-7 cells (African green monkey kidney cells) transfected with the rat neuropeptide Y Y5 receptor cDNA was tested. It appeared that several analogues displayed different inhibition constants (K_i) in the two binding assays, more especially *N*-α-acetyl-peptide YY-(22–36) which was 1200 × more potent in the crypt cell binding assay than in the recombinant neuropeptide Y Y5 receptor binding assay. These data support that the intestinal crypt peptide YY receptor is not a Y5 receptor.

Keywords: Peptide YY receptor; Neuropeptide Y receptor; (Rat); Intestine

1. Introduction

Neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) are the members of a family of structurally related peptides (Larhammar, 1996). They have a broad range of biological actions which are mediated by the so-called Y receptors which comprise at least six subtypes from Y1 to Y6 (Blomqvist and Herzog, 1997). We recently showed that Y receptor mRNAs including Y1, Y2, Y4 and Y5 are expressed in the rat small intestine or colon (Goumain et al., 1998) where peptide YY, neuropeptide Y and pancreatic polypeptide exert potent inhibition of fluid and electrolyte secretion (Cox et al., 1988; Laburthe, 1990; Souli et al., 1997). More particularly, the Y5 receptor mRNA is present in small intestinal crypt cells but not in colon epithelium (Goumain et al., 1998), a distribution that is similar to that of [^{125}I]peptide YY binding in gut epithelia (Laburthe et al., 1986; Voisin et al., 1990). This prompted us to consider the possibility that intestinal epithelial peptide YY receptors and neuropeptide Y Y5 receptors may be the same entity. Indeed, the receptor responsible for peptide YY binding in rat small intestinal epithelium has been characterised functionally (Laburthe et al., 1986; Servin et al., 1989) and at the biochemical level by cross-linking experiments and after solubilisation (Voisin et al., 1991), but its molecular nature remains elusive since it has not been cloned yet.

In the present work, we have analysed the distribution of neuropeptide Y Y5 receptor mRNA and that of [^{125}I]peptide YY binding in epithelial cell isolated from rat small intestine crypts and villi and also from colon. Moreover, we have compared the pharmacological profile of [^{125}I]peptide YY binding to rat intestinal crypt cells and to the recombinant rat Y5 receptor transfected into COS-7 cells (African green monkey kidney cells) with a series of selected peptide YY, neuropeptide Y or pancreatic

* Corresponding author. Tel.: +33-1-44-85-61-35; Fax: +33-1-44-85-61-24; E-mail: laburthe@bichat.inserm.fr

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00757-2

polypeptide analogues. Our data show that the pharmacological profiles of the rat intestinal crypt cell receptor for peptide YY and of neuropeptide Y Y5 receptors are clearly different.

2. Materials and methods

2.1. Generation of a rat Y5 clone by RT-PCR (reverse transcription polymerase chain reaction) and transient transfection protocol

Rat brain DNAse-treated RNA was extracted with the RNAxel reagent (Eurobio, Les Ulis, France) following the protocol of the manufacturer (Chomczynski and Sacchi, 1987). A total of 10 μg of RNA were reverse-transcribed using oligo(dT_{15}) (Promega, Charbonnières, France) and M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase (Life Technologies, Cergy, France) and five μg of the resulting cDNA mixture were submitted to PCR (Polymerase Chain Reaction) using specific primers. Non coding sense (5′-CCC GAG GAT TTT AGT ATG GAG-3′) and antisense (5′-TAT CAC TCA ACT TAT TAC CAA-3′) primers were used for PCR on rat brain cDNA using the following conditions: 1 min at 94°C, 1 min at 45°C, and 90 s at 72°C for 30 cycles using *Taq* polymerase (Promega). A PCR product with the expected full-length rat Y5 cDNA size (1561 bp) was cloned using the ligator kit (R&D Systems, Abingdon, UK) and sequenced with a DNA sequencing kit (Amersham, Les Ulis, France). It displayed 100% identity with the sequence of the cloned rat Y5 receptor cDNA (Gerald et al., 1996; Hu et al., 1996). COS-7 cells were transiently transfected by using an electroporation method as described (Couvineau et al., 1996). Cells were harvested in phosphate-buffered saline 48 h after transfection and particulate membranes were prepared as described (Couvineau et al., 1996).

2.2. Tissue preparation

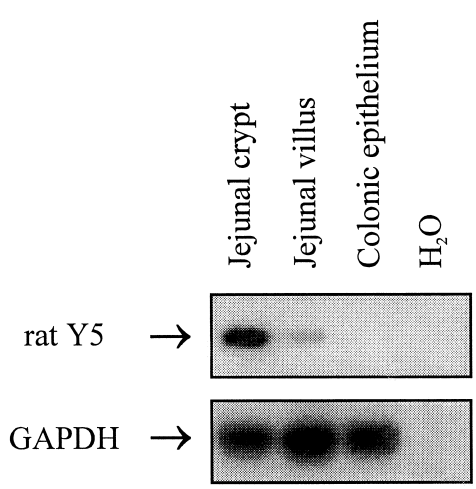
Three-month-old male Wistar rats fed ad libitum were used in this study. Jejunum and colon epithelial cells were prepared as described (Laburthe et al., 1986). Jejunal crypt cells were separated from villus cells by shaking the everted jejunum for successive periods in a dispersing solution containing EDTA as previously described in detail (Voisin et al., 1990). Membranes were prepared from crypt cells as described (Laburthe et al., 1986).

2.3. RT-PCR analysis of intestinal tissues

Isolated cells (crypt, villus, colon) were homogenized in RNAxel solution (Eurobio) and extraction of RNA was performed according to the protocol suggested by the supplier. All RNA preparations were treated with RNAse-free DNAse (Promega) and reverse transcribed as described above. For neuropeptide Y Y5 receptor, PCR was performed using the following conditions: 1 min at 94°C, 1 min at 64°C, and 1 min at 72°C. Primers used are sense 5′-CCA GGC AAA AAC CCC CAG CAC-3′ at position 834, and antisense 5′-GGC AGT GGA TAA GGG CTC TCA-3′ at position 1357. PCR products were analysed by electrophoresis on 1% agarose gel, capillary transferred to Hybond membrane (Amersham) overnight and submitted to Southern hybridisation with rat Y5 receptor [^{32}P]-labelled cDNA fragment 834–1357. Primers for glyceraldehyde-3-phosphate-deshydrogenase (GAPDH), taken as a control for a house-keeping protein, were used as described (Maoret et al., 1994).

2.4. Binding assay

Binding of [^{125}I]peptide YY to membranes prepared from isolated cells or transfected cells was carried out as described (Voisin et al., 1990). All binding data were analysed using the LIGAND computer program (Munson and Rodbard, 1980). The constant K_i for the inhibition of [^{125}I]peptide YY binding by unlabelled peptides were calculated from the concentration of unlabelled peptide that produce 50% inhibition (IC_{50}) of the specific [^{125}I]peptide



	Jejunal crypt	Jejunal villus	Colonic epithelium	H_2O
Peptide YY Binding (fmol/mg prot.)	130 ±32	13 ±4	N.D.	

Fig. 1. PCR-based detection of expression of neuropeptide Y Y5 receptor mRNA and peptide YY binding in jejunum epithelium (crypt and villus) and colon epithelium. Top: cDNAs from indicated tissues were used as templates for PCR reactions using specific primers (see Section 2). Southern hybridisation with specific [^{32}P]-labelled probes are shown. Bottom: Binding capacities were calculated by Scatchard analysis of the inhibition of [^{125}I]peptide YY binding by unlabelled peptide YY. Data show means ± S.E.M. (three experiments). The dissociation constants (K_d) in jejunal crypts and villi were similar, e.g., 0.1 nM. Abbreviations: GAPDH, Glyceraldehyde-3-phosphate-deshydrogenase; N.D., not detected.

Table 1
Ability of peptide YY, neuropeptide Y, pancreatic polypeptide and analogues to inhibit [^{125}I]peptide YY binding to membranes prepared from rat jejunal crypts and COS-7 cells transfected with the rat neuropeptide Y Y5 receptor cDNA

	Jejunal crypt K_i (nM)	Recombinant Y5 K_i (nM)	K_i(Y5)/K_i(crypt)
Neuropeptide Y	1.3 ± 0.1	1.5 ± 0.3	1.2
Neuropeptide Y-(3–36)	2.3 ± 0.3	3.7 ± 0.5	1.6
Neuropeptide Y-(13–36)	3.5 ± 0.5	26 ± 4	7.4
[Leu^{31}, Pro^{34}]neuropeptide Y	59 ± 9	4.8 ± 0.7	0.08
[D-Trp^{32}]neuropeptide Y	36 ± 6	48 ± 11	1.3
Peptide YY	0.3 ± 0.1	0.9 ± 0.2	3.0
Peptide YY-(3–36)	0.8 ± 0.1	6.3 ± 0.7	7.8
N-α-acetyl-peptide YY-(22–36)	1.3 ± 0.1	1518 ± 436	1168
Rat pancreatic polypeptide	2040 ± 354	170 ± 34	0.08
Human pancreatic polypeptide	1789 ± 205	23 ± 7	0.012

K_i values were calculated as described in Section 2. Data show means ± S.E.M. (three experiments).

YY binding using the following relation: $K_i = IC_{50} \times [K_d/(K_d + L)]$ where K_d is the dissociation constant and L the concentration of [^{125}I]peptide YY. Rat neuropeptide Y-(1–36), porcine neuropeptide Y-(3–36), rat neuropeptide Y-(13–36), rat [Leu^{31}, Pro^{34}]neuropeptide Y, rat [D-Trp^{32}]neuropeptide Y, porcine peptide YY-(1–36), porcine peptide YY-(3–36), rat pancreatic polypeptide and human pancreatic polypeptide were purchased from Neosystem (Strasbourg, France) or Peninsula Laboratories (Belmont, CA, USA). Rat *N*-α-acetyl-peptide YY-(22–36) was synthesised as described (Balasubramaniam et al., 1993).

3. Results

After Southern blotting with rat neuropeptide Y Y5 receptor [^{32}P]-labelled cDNA of the products obtained after RT-PCR using specific Y5 receptor primers, a labelled band of the expected size (524 bp) was observed in rat jejunal crypt cells whereas a very faint one of the same size was detectable in rat jejunal villus cells (Fig. 1). In sharp contrast, no band could be detected in rat colonic epithelial cells. The presence of PCR products for GAPDH, taken as a control for a house-keeping gene, was observed in all cell preparations. Moreover, when water was added instead of cDNA preparations, no PCR products could be detected for either Y5 receptor or GAPDH primers assessing for the absence of contamination during the RT-PCR process. These data supported that Y5 receptor mRNAs were mainly expressed in rat jejunal crypt cells with very few or no expression in rat jejunal villus cells or colonic epithelial cells, respectively. This distribution is identical to the distribution of [^{125}I]peptide YY binding in isolated epithelial cells from rat crypt, villus and colon. Indeed, Scatchard analysis of binding data indicated the presence of 10 × more abundant binding sites in jejunal crypt cells than in jejunal villus cells with the absence of detectable binding sites in colonic epithelium (Fig. 1).

In this context, it could be hypothesised that [^{125}I]peptide YY binding to intestinal crypts was related to its interac-

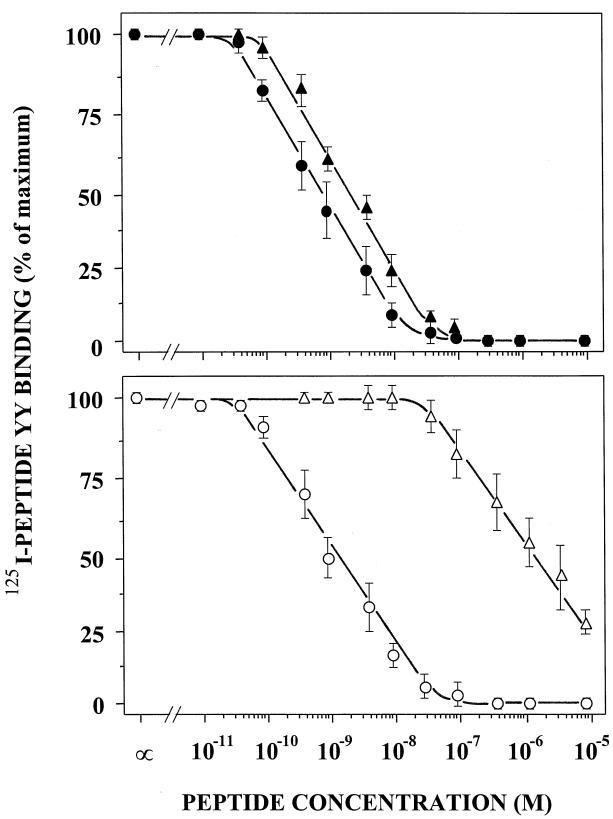


Fig. 2. Inhibition of [^{125}I]peptide YY binding by unlabelled peptide YY and *N*-α-acetyl-peptide YY-(22–36) in membranes prepared from crypt cells (top) and COS-7 cells transfected with the Y5 receptor (bottom). [^{125}I]peptide YY was incubated with increasing concentrations of peptide YY (circles) or *N*-α-acetyl-peptide YY-(22–36) [triangles]. [^{125}I]peptide YY binding was expressed as percent of maximal specific binding in the absence of unlabelled competitor. Hill coefficients n_H were calculated from competition curves obtained with peptide YY or *N*-α-acetyl-peptide YY-(22–36). In crypt cell membranes, they were 0.93 ($r = 0.99$) and 0.94 ($r = 0.99$), respectively. In membranes from COS-7 cells transfected with the Y5 receptor, they were 0.96 ($r = 0.97$) and 0.91 ($r = 0.95$), respectively. Nonspecific binding and binding parameters were determined using the LIGAND computer program (Munson and Rodbard, 1980). Data show means ± S.E.M. (three experiments).

tion with a Y5 receptor. In order to test this hypothesis, we compared the pharmacological profiles of [^{125}I]peptide YY binding to membranes prepared from isolated rat intestinal crypts and from COS-7 cells transfected with the rat Y5 receptor cDNA (Table 1). As previously described (Laburthe et al., 1986), the crypt cell receptor was slightly peptide YY-preferring with a 4-fold higher affinity for peptide YY than for neuropeptide Y. It had a very low affinity for rat and human pancreatic polypeptide with a K_i of 2040 nM and 1789 nM, respectively. The recombinant rat Y5 receptor had a similar high affinity for peptide YY and neuropeptide Y but presented a higher affinity for human pancreatic polypeptide ($K_i = 23$ nM) than for rat pancreatic polypeptide ($K_i = 170$ nM), as described (Gerald et al., 1996). The test of several peptide YY or neuropeptide Y fragments or analogues revealed further pharmacological differences (Table 1). This includes neuropeptide Y-(13–36), peptide YY-(3–36) and [Leu^{31}, Pro^{34}]neuropeptide Y which have 7 × lower, 7 × lower and 12 × higher K_i in crypt cells than in COS-7 cells transfected with the Y5 receptor, respectively. Finally, *N*-α-acetyl-peptide YY-(22–36) unambiguously discriminated between the two receptor binding assays (Fig. 2) with a dramatic difference of K_i in rat crypt cells and COS-7 cells transfected with the Y5 receptor i.e., 1.3 and 1518 nM, respectively. Other analogues including neuropeptide Y-(3–36) and [D-Trp^{32}]neuropeptide Y had similar K_i in the two binding assays.

4. Discussion

The characterisation of specific [^{125}I]peptide YY binding to rat small intestinal epithelial cells (Laburthe et al., 1986) had been a very early description of receptors for peptide YY and neuropeptide Y. Since intestinal cells have a slightly higher affinity for peptide YY than for neuropeptide Y, it had been proposed that this intestinal receptor could be defined as a peptide YY-preferring receptor (Laburthe et al., 1986; Laburthe, 1990). This was in good agreement with further reports indicating that peptide YY was slightly more potent than neuropeptide Y in inhibiting electrolyte secretion in rat small intestine (Cox et al., 1988) and cAMP production in isolated rat small intestinal epithelial cells (Servin et al., 1989). This receptor negatively coupled to cAMP production was shown to be present in small intestinal epithelium and not in colonic epithelium (Laburthe et al., 1986; Servin et al., 1989). Moreover, it appears to be expressed mainly in small intestinal crypt cells and to be quenched when cells stop to divide and differentiate into mature villus cells (Voisin et al., 1990). However, the exact nature of this receptor has remained elusive since it has not been cloned yet.

Following the cloning of several receptor subtypes for pancreatic polypeptide-fold peptides (see Blomqvist and Herzog, 1997 for a review), we were recently able to show that Y1, Y2, Y4 and Y5 receptor mRNAs are expressed in rat small intestine or colon (Goumain et al., 1998). The distribution of Y1, Y2 and Y4 receptor mRNAs is quite different from that of [^{125}I]peptide YY binding in small and large intestinal epithelia. However, the distribution of Y5 receptor mRNAs (Goumain et al., 1998) was shown to be reminiscent of that of [^{125}I]peptide YY binding (Laburthe et al., 1986; Voisin et al., 1990). Since rat Y5 receptors have a high affinity for both peptide YY and neuropeptide Y (Hu et al., 1996), they could therefore represent the elusive intestinal peptide YY receptor. In the present paper, we further demonstrate the Y5 receptor mRNAs and [^{125}I]peptide YY binding have a very similar pattern of distribution in small and large intestine epithelium (see Fig. 1) e.g., absence in colon and expression almost restricted to small intestinal crypts. However, we provide strong pharmacological evidence supporting that [^{125}I]peptide YY binding sites in rat small intestinal crypt cells are not Y5 receptors. Indeed, by comparing the pharmacological profiles of [^{125}I]peptide YY binding to the recombinant rat neuropeptide Y Y5 receptor and to receptors in rat jejunal crypt cells, it was found that several peptides have a > 10-fold higher affinity for recombinant Y5 receptors than for crypt cell receptors including [Leu^{31}, Pro^{34}]neuropeptide Y, rat and human pancreatic polypeptide (see Table 1). The reverse was also true with peptides having a higher affinity for crypt cell receptors than for recombinant Y5 receptors including neuropeptide Y-(13–36), peptide YY-(3–36) and *N*-α-acetyl-peptide YY-(22–36). This was especially evident for *N*-α-acetyl-peptide YY-(22–36) which exhibits a > 1000-fold higher affinity for crypt cell receptors than for recombinant neuropeptide Y Y5 receptors. In this context, it is worth pointing out that *N*-α-acetyl-peptide YY-(22–36) which has a high affinity for intestinal crypt receptors was also shown to be very potent in inhibiting cAMP production in small intestinal cells in vitro (Servin et al., 1989) and in inhibiting fluid secretion in rat jejunum in vivo (Balasubramaniam et al., 1993; Souli et al., 1997). These data strongly suggest that not only intestinal crypt cell receptors for peptide YY and neuropeptide Y are not Y5 receptors but also support the idea that Y5 receptor contribute very little, if any, in peptide YY binding, peptide YY-induced inhibition of cAMP production and peptide YY-induced inhibition of fluid secretion in rat jejunum. This is in line with the fact that peptide YY binds to a homogenous population of noninteracting receptors in rat small intestinal crypts as supported by Scatchard analysis (Voisin et al., 1990) and a Hill coefficient close to 1 (Balasubramaniam et al., 1993; Fig. 2).

In conclusion, the present work demonstrates that the intestinal crypt cell peptide YY receptor is not a neuropeptide Y Y5 receptor and suggests that Y5 receptors, although expressed in small intestinal crypts, at least at the mRNA level, probably do not contribute predominantly to the inhibitory effect of peptide YY and neuropeptide Y on

cAMP production and fluid secretion by small intestinal epithelial cells. Since the distribution of peptide YY binding and peptide YY-induced inhibition of cAMP production is also different from that of Y1, Y2 and Y4 receptor mRNAs in small and large intestinal epithelium (Goumain et al., 1998), it may be further suggested that the elusive peptide YY-preferring receptor expressed in rat small intestinal crypts (Laburthe, 1990) correspond to a still not cloned subtype of Y receptors. Current hypothesis on Y receptor subtypes does favour this view (Blomqvist and Herzog, 1997).

References

Balasubramaniam, A., Cox, H.M., Voisin, T., Laburthe, M., Stein, M., Fisher, J.E., 1993. Structure-activity studies of peptide YY (22–36): *N*-alpha-Ac-[Phe^{27}]-PYY(22–36), a potent antisecretory peptide in rat jejunum. Peptides 14, 1011–1016.

Blomqvist, A.G., Herzog, H., 1997. Y receptor subtypes—how many more?. Trends Neurosci. 20, 294–298.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Couvineau, A., Rouyer-Fessard, C., Maoret, J.J., Gaudin, P., Nicole, P., Laburthe, M., 1996. Vasoactive intestinal peptide $(VIP)_1$ receptor. J. Biol. Chem. 271, 12795–12800.

Cox, H.M., Cuthbert, A.M., Hakanson, R., Wahlestedt, C., 1988. The effect of neuropeptide Y and peptide YY on electrogenic ion transport in rat intestinal epithelia. J. Physiol. (London) 398, 65–80.

Gerald, C., Walker, M.W., Criscione, L., Gustafson, E.L., Batzl-Hartmann, C., Smith, K.E., Vaysse, P., Durkin, M.M., Laz, T.M., Linemeyer, D.L., Schaffhauser, A.O., Whitebread, S., Hofbauer, K.G., Taber, R.I., Branchek, T.A., Weinshank, R.L., 1996. A receptor subtype involved in neuropeptide Y-induced food intake. Nature 382, 168–171.

Goumain, M., Voisin, T., Lorinet, A.M., Laburthe, M., 1998. Identification and distribution of mRNA encoding the Y1, Y2, Y4 and Y5 receptors for peptides of the PP-fold family in the rat intestine and colon. Biochem. Biophys. Res. Commun. 247, 52–56.

Hu, Y., Bloomqvist, B.T., Cornfield, L.J., DeCarr, L.B., Flores-Riveros, J.R., Friedman, L., Jiang, P., Lewis-Higgins, L., Sadlowski, Y., Schaefer, J., Velazquez, N., McCaleb, M.L., 1996. Identification of a novel hypothalamic neuropeptide Y receptor associated with feeding behavior. J. Biol. Chem. 271, 26315–26319.

Laburthe, M., 1990. Peptide YY and neuropeptide Y in the gut: availability, biological actions and receptors. Trends Endocr. Metab. 1, 168–174.

Laburthe, M., Chenut, B., Rouyer-Fessard, C., Tatemoto, K., Couvineau, A., Servin, A., Amiranoff, B., 1986. Interaction of peptide YY with rat intestinal epithelial plasma membranes: binding of the radioionated peptide. Endocrinology 118, 1917.

Larhammar, D., 1996. Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. Regul. Pept. 62, 1–11.

Maoret, J.J., Pospaï, D., Rouyer-Fessard, C., Couvineau, A., Laboisse, C., Voisin, T., Laburthe, M., 1994. Neurotensin receptor and its mRNA are expressed in many human colon cancer cell lines but not in normal colonic epithelium: binding studies and RT-PCR experiments. Biochem. Biophys. Res. Commun. 203, 465–471.

Munson, P.J., Rodbard, D., 1980. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 197, 220–239.

Servin, A.L., Rouyer-Fessard, C., Balasubramaniam, A., Saint-Pierre, S., Laburthe, M., 1989. Peptide YY and neuropeptide Y inhibit vasoactive intestinal peptide-stimulated adenosine 3′,5′-monophosphate production in rat small intestine: structural requirements of peptides for interacting with peptide YY-preferring receptors. Endocrinology 124, 692–700.

Souli, A., Chariot, J., Voisin, T., Presset, O., Tsocas, A., Balasubramanian, A., Laburthe, M., Rozé, C., 1997. Several receptors mediate the antisecretory effect of peptide YY, neuropeptide Y and pancreatic polypeptide on VIP-induced fluid secretion in the rat jejunum in vivo. Peptides 18, 551–557.

Voisin, T., Rouyer-Fessard, C., Laburthe, M., 1990. Distribution of the common peptide YY/neuropeptide Y receptor along rat intestinal villus–crypt axis. Am. J. Physiol. 258, G753–G759.

Voisin, T., Couvineau, A., Rouyer-Fessard, C., Laburthe, M., 1991. Solubilisation and hydrodynamic properties of active peptide YY receptor from rat jejunal crypts. J. Biol. Chem. 266, 10762–10767.