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Vasoactive intestinal peptide modification at position 22 allows discrimination between receptor subtypes

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Abstract

Secretin and growth hormone releasing factor (GRF) have a weak affinity for VIP (vasoactive intestinal peptide)/PACAP (pituitary adenylate cyclase activating polypeptide) receptors, but discriminate between VIP_1/PACAP and VIP_2/PACAP receptors. This previously allowed us to develop modified secretin and GRF derivatives as high affinity and highly selective VIP_1/PACAP receptor ligands. We tested the hypothesis that the presence of a Gln residue at position 24 and a Leu residue at position 22 was responsible for their VIP_1/PACAP receptor selectivity. [Gln^{24}]VIP was not different from VIP but [Leu^{22}]VIP had a 100-fold lower affinity for VIP_2/PACAP receptors as compared to VIP_1/PACAP receptors. The substitution of Tyr^{22} by Phe^{22} in VIP had no significant effect on the recognition of both receptors but [Ala^{22}]VIP had a reduced affinity for the VIP_2/PACAP receptor. This indicated that an aromatic residue at position 22 of VIP was required for a high affinity for the VIP_2/PACAP receptor but not for the VIP_1/PACAP receptor. © 1998 Elsevier Science B.V.

Keywords: VIP (vasoactive intestinal peptide) analog; VIP_1/PACAP receptor; VIP_2/PACAP receptor

1. Introduction

VIP (vasoactive intestinal peptide) recognizes two distinct high-affinity receptors, named the VIP_1/PACAP (pituitary adenylate cyclase activating polypeptide) and the VIP_2/PACAP receptors, that have been cloned in rats (Ishihara et al., 1992; Lutz et al., 1993) and humans (Couvineau et al., 1994; Svoboda et al., 1994). The mouse VIP_2/PACAP receptor has also been cloned (Inagaki et al., 1994). Both receptor subtypes are coupled to G_s proteins and their occupancy by agonists stimulates adenylate cyclase activity. VIP and PACAP are equally potent on both receptors and do not exhibit a significant preference for one receptor subtype. The tissue distribution of the mRNA coding for each receptor subtype has been investigated by in situ hybridization (Usdin et al., 1994) and has revealed a tissue-specific expression.

We recently found that the cyclic peptide analogue of VIP, RO 25-1553 (O'Donnell et al., 1994a,b) (see Fig. 1), is a highly selective agonist ligand for the VIP_2/PACAP receptor subtype (Gourlet et al., 1997a). Secretin and growth hormone releasing factor (GRF) have poor affinity for VIP_1/PACAP receptors, and almost no affinity for VIP_2/PACAP receptors (Gourlet et al., 1997b). We took advantage of this property to develop selective agonists (Gourlet et al., 1997b) and a selective antagonist (Gourlet et al., 1997c) for the VIP_1/PACAP receptor. The location of the two receptor subtypes in the rat brain was studied with these new tools (Vertongen et al., 1997) and confirmed the results obtained by in situ hybridization.

Comparison of the amino acid sequences of non-selective VIP/PACAP receptor ligands and of selective VIP_1/PACAP receptor ligands (Fig. 1) suggested that the amino acid residues at position 22 and 24 could be important for receptor discrimination: the selective VIP_1/PACAP receptor ligands had a leucine (L) residue instead of a tyrosine (Y) residue at position 22 and a glutamine (Q) residue instead of alanine (A) or asparagine (N) at position 24 of PACAP and VIP, respectively.

We prepared [Leu^{22}]VIP and [Gln^{24}]VIP and evaluated their ability to interact with rat and human VIP_1/PACAP

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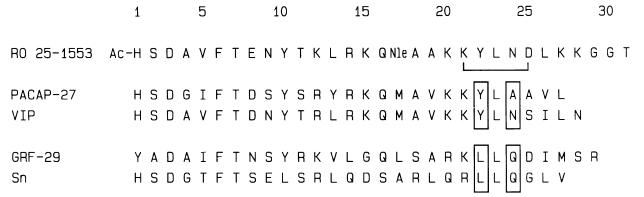

Fig. 1. Amino acid sequences of the VIP_2 selective peptide, RO 25-1553 (O'Donnell et al., 1994a,b), of the two non-selective agonists (common PACAP and common VIP) and of two agonists that preferred VIP_1/PACAP receptors to VIP_2/PACAP receptors (human GRF and porcine secretin). The boxes indicate the residues that are identical in the two VIP_1/PACAP-selective ligands, and different from the corresponding residues in the two non-selective ligands.

and VIP_2/PACAP receptors. [Leu^{22}]VIP but not [Gln^{24}]VIP had a reduced affinity for the VIP_2/PACAP receptor. Similar results were obtained with [Ala^{22}]VIP but not with [Phe^{22}]VIP, indicating the crucial role of an aromatic residue at position 22 of VIP for a high-affinity interaction with the VIP_2/PACAP receptor.

2. Materials and methods

2.1. Cell lines

Four Chinese hamster ovary (CHO) cell lines expressing recombinant receptors were used for receptor characterization. The cell lines expressing the rat VIP_1/PACAP (850 fmol/mg of receptor of membrane protein) and the human VIP_2/PACAP (200 fmol receptor mg^{-1} membrane protein) receptors were previously described (Ciccarelli et al., 1994; Svoboda et al., 1994). The cell line expressing the rat VIP_2/PACAP receptor (500 fmol receptor/mg membrane protein) was kindly provided by Dr. E.M. Lutz from the MRC Brain Metabolism Unit, Edinburgh, and the cell line expressing the human VIP_1 receptor was established by electroporation transfection of the cDNA cloned in pcDNA 3 plasmid (Invitrogen) and selection by subcloning of a CHO cell clone expressing around 800 fmol of receptor/mg membrane protein. This clone has already been used in previous studies (Gourlet et al., 1997b,c).

All the cell lines were cultured in the same medium, as described previously (Ciccarelli et al., 1994).

2.2. Membrane preparation and receptor identification

Transfected CHO cells were harvested with a rubber policeman and pelleted by low-speed centrifugation; the supernatant was discarded and the cells were lysed in 1 mM $NaHCO_3$ solution and immediately frozen in liquid nitrogen.

After thawing, the lysate was first centrifuged at 4°C for 10 min at $400 \times g$ and the supernatant was further centrifuged at $20\,000 \times g$ for 10 min. The pellet, resuspended in 1 mM $NaHCO_3$, was used immediately as a crude membrane fraction.

Binding was performed as described (Ciccarelli et al., 1994; Vertongen et al., 1997), using [^{125}I]VIP for characterization of the rat and human VIP_1 receptor and [^{125}I]RO 25-1553 for characterization of rat and human VIP_2 receptors. [^{125}I]RO 25-1553 had a 3-fold higher affinity than [^{125}I]VIP for VIP_2/PACAP receptors, so that the results obtained with this tracer were technically more satisfactory. The competition curves obtained with [^{125}I]VIP and [^{125}I]RO 25-1553 were superimposable (Vertongen et al., 1997). In all cases, non-specific binding was defined as the residual binding in the presence of 1 μM VIP. Binding was performed at 37°C in a 20-mM Tris–maleate, 2 mM $MgCl_2$, 0.1 mg ml^{-1} bacitracin, 1% bovine serum albumin (pH 7.4) buffer. Bound radioactivity was separated from free by filtration through glass fibre GF/C filters presoaked for 24 h in 0.01% polyethyleneimine and rinsed three times with a 20 mM (pH 7.4) sodium phosphate buffer containing 1% bovine serum albumin. Adenylate cyclase activity was determined by the procedure of Salomon et al. (1974). Membrane protein (3–15 μg) was incubated in a total volume of 60 μl containing 0.5 mM [α-^{32}P]ATP, 10 μM GTP, 5 mM $MgCl_2$, 0.5 mM EGTA, 1 mM cAMP, 1 mM theophylline, 10 mM phospho(enol)pyruvate, 30 μg/ml pyruvate kinase and 30 mM Tris–HCl at a final pH of 7.5. The reaction was

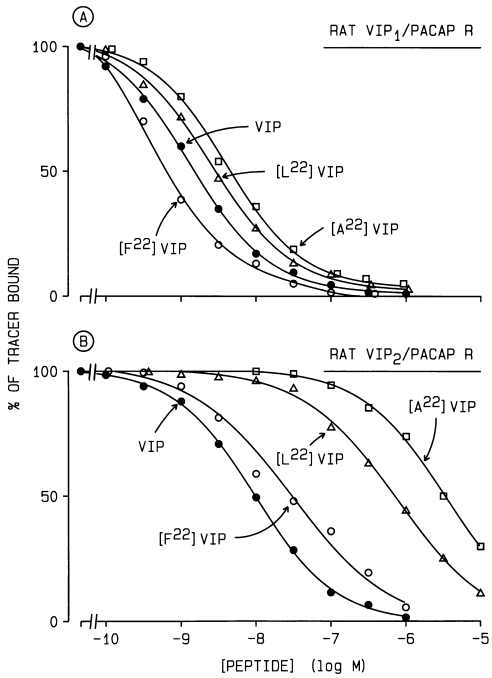

Fig. 2. Inhibition of [^{125}I]VIP binding to membranes from CHO cells expressing the rat VIP_1/PACAP receptor (upper panel) and of [^{125}I] RO 25-1553 binding to membranes from CHO cells expressing the rat VIP_2/PACAP receptor (lower panel) by VIP (●), [A^{22}]VIP ([Ala^{22}]VIP) (□), [F^{22}]VIP ([Phe^{22}]VIP) (○) and [L^{22}]VIP ([Leu^{22}]VIP) (△). The results are the means of three experiments.

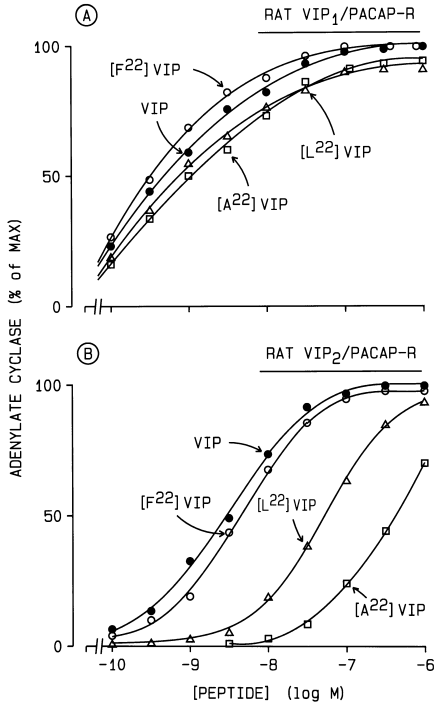

Fig. 3. Dose–effect curves for adenylate cyclase activation of membranes from CHO cells expressing the rat VIP_1/PACAP receptor (upper panel) and the rat VIP_2/PACAP receptor (lower panel) by VIP (●), [A^{22}]VIP ([Ala^{22}]VIP) (□), [F^{22}]VIP ([Phe^{22}]VIP) (○) and [L^{22}]VIP ([Leu^{22}]VIP) (△). The adenylate cyclase activity increased from 40 to 300 and 30 to 240 pmol cAMP/min per mg protein in the presence of 1 μM VIP in membrane from cells expressing rat VIP_1/PACAP and rat VIP_2/PACAP receptors, respectively. The results, expressed as % of the maximal value obtained in the presence of 1 μM VIP, are the means of three experiments.

initiated by adding membranes and was terminated after a 15-min incubation at 37°C by adding 0.5 ml of a 0.5% sodium dodecylsulfate solution containing 0.5 mM ATP, 0.5 mM cAMP and 20 000 cpm [8-^{3}H]cAMP. cAMP was separated from ATP by two successive chromatography steps on Dowex 50 W × 8 and neutral alumina.

2.3. Peptide synthesis

The peptides were synthesized as C-terminal amides by solid phase methodology on an automated Applied Biosystems apparatus (Foster City, CA), using the 9-fluorenylmethoxy carbonyl strategy (Ambrosius et al., 1989). They were purified by reversed-phase chromatography on Jordi-Gel DVB 300 Å (10 × 1 cm) and by ion exchange chromatography on Mono S HR 5/5. Peptide purity (> 95%) was assessed by capillary electrophoresis and the sequence conformity was verified by sequencing and electrospray mass spectrometry.

3. Results

3.1. Interaction of [Gln^{24}]VIP and [Leu^{22}]VIP with VIP_1/PACAP- and VIP_2/PACAP receptors

As mentioned in the introduction, [Gln^{24}]VIP and [Leu^{22}]VIP were synthesized to test the hypothesis, based on amino acid sequences comparison (Fig. 1), that the introduction of a glutamine at position 24 and a leucine at position 22 would promote selectivity for the VIP_1/PACAP receptor subtype. [Gln^{24}]VIP was not different from VIP, in terms of both its affinity for the rat- and human recombinant VIP_1/PACAP and VIP_2/PACAP receptors (Table 1), and its ability to stimulate adenylate cyclase activity (results not shown). [Leu^{22}]VIP was indistinguishable from VIP at both rat and human VIP_1/PACAP receptors but was 100-fold less potent than VIP at the VIP_2/PACAP receptors (Figs. 2 and 3 and Table 1).

3.2. Interaction of [Phe^{22}]VIP and [Ala^{22}]VIP with VIP_1/PACAP- and VIP_2/PACAP receptors

These two analogues were synthesized to test whether the low affinity of [Leu^{22}]VIP for the VIP_2/PACAP receptor was due to the lack of an aromatic function or to its replacement by a highly hydrophobic residue.

[Phe^{22}]VIP was similar to VIP (which has a Tyr residue at position 22) and [Ala^{22}]VIP was comparable to [Leu^{22}]VIP in all the systems tested. The affinity of [Ala^{22}]VIP for the VIP_1/PACAP human receptor was even lower than that of [Leu^{22}]VIP (Figs. 2 and 3 and Table 1). [Ala^{22}]- and [Phe^{22}]VIP were full agonists on adenylate cyclase stimulation (Figs. 2 and 3 and data not shown).

Table 1
IC_{50} values (in nM) for binding of VIP, [Gln^{24}]VIP, [Leu^{22}]VIP, [Phe^{22}]VIP and [Ala^{22}]VIP to rat and human VIP_1/PACAP and VIP_2/PACAP receptors

Peptide tested	IC_{50} (nM)			
	Rat		Human	
	VIP_1/PACAP	VIP_2/PACAP	VIP_1/PACAP	VIP_2/PACAP
GRF[a]	80	30 000	100	30 000
Secretin[a]	300	30 000	1500	5000
VIP	2	10	2	10
[Gln^{24}]VIP	2	3	1	3
[Leu^{22}]VIP	3	800	8	1000
[Phe^{22}]VIP	1	20	1	10
[Ala^{22}]VIP	4	3000	10	1000

The IC_{50} values were established, using the LIGAND programme, from inhibition of tracer binding by increasing concentrations of unlabelled peptides. The results were the means of three determinations and the standard deviation (S.D.) was less than 0.1 log unit in all cases.
[a] Previously published data (Gourlet et al., 1997b).

4. Discussion

Owing to the broad distribution of the VIP receptors in the pulmonary systems, gastrointestinal tract, genito-urinary tract, as well as in vessels, heart, endocrine glands, brain and immunocompetent cells, the potential applications of VIP as a drug are large, but the expected undesired effects are numerous. The recent discovery that VIP interacts with two distinct receptor subclasses, with different tissue and cell distributions, and the development of selective agonists (Usdin et al., 1994; Gourlet et al., 1997a,b,c) has increased interest in pharmacological applications of this class of peptides. At present, relatively little is known about the structural basis for the high-affinity interaction of VIP with each receptor class.

We previously established that the carboxy terminus was important for VIP_1–VIP_2 receptor discrimination: analogues truncated at the carboxy terminus had a decreased affinity for both VIP receptors but this effect was more pronounced for the VIP_2/PACAP receptor than for the VIP_1/PACAP receptor (Gourlet et al., 1996a). Conversely, analogues that were carboxy-terminally extended had a higher affinity for the VIP_2/PACAP receptor than for the VIP_1/PACAP receptor (Gourlet et al., 1996b). Studies conducted before the molecular cloning of the VIP_1- and VIP_2/PACAP receptors on SUP-T1 cells (that express the VIP_2/PACAP receptor: Robberecht et al., 1989; Svoboda et al., 1994; Robberecht et al., 1996) and on rat liver and pancreatic membranes (that express the VIP_1/PACAP receptor: Robberecht et al., 1987) suggested that amino-terminally modified VIP analogues also had the ability to discriminate between the two receptor subclasses: [D-Ala^4]VIP and [D-Phe^4]VIP had a 100-fold higher affinity for VIP_1 receptors than for VIP_2/PACAP receptors. These results were recently confirmed with the recombinant receptors (Gourlet et al., 1997d).

The present data indicate that the side chain of the amino acid residue at position 22 is also of importance for receptor discrimination: an aromatic residue (tyrosine or phenylalanine) is necessary for a high-affinity interaction with the VIP_2/PACAP receptor but not with the VIP_1/PACAP receptor. Indeed, the introduction of a hydrophobic residue (leucine or alanine) in that position markedly reduced the affinity of VIP for the VIP_2/PACAP receptor only.

The observation that [Ala^{22}]VIP is a full agonist with a high affinity for human- and rat-VIP_1/PACAP receptors suggests that a reinterpretation of previous data from O'Donnell et al., 1991 is necessary. These authors synthesized VIP analogues systematically modified with single alanine substitutions ('Ala-Scan') and tested the peptides in binding assays with guinea pig and human lung preparations, in bioassays measuring the relaxation of guinea pig tracheal preparations, and, in vivo, by peptide instillation into the trachea. In all these experimental models, the [Ala^{22}]VIP analogue was 50- to 400-fold less potent than the corresponding [Tyr^{22}] peptide. This suggests that the VIP_2/PACAP receptors were the main contributors to the VIP-induced broncho-tracheal relaxation. This conclusion is in line with the finding that the stable VIP analogue RO 25-1553, a potent broncho-relaxing agent (O'Donnell et al., 1994a), is a highly selective VIP_2/PACAP receptor agonist (Gourlet et al., 1997a). In membranes from total lung homogenates, binding and functional studies (Robberecht et al., 1982, 1988) however indicate a major contribution of VIP_1/PACAP receptors, which could be located on the epithelial cells (Usdin et al., 1994). Thus, the present results, besides offering new perspectives for the design of selective analogues, also focus the attention on the fact that the previously published VIP 'Ala-Scan' (O'Donnell et al., 1991) was probably tested on VIP_2/PACAP receptors only.

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References

Ambrosius, D., Casaretto, M., Gerardy-Schahn, R., Saunders, D., Brandenburg, D., Zahn, H., 1989. Peptide analogues of the anaphylatoxin C3a; syntheses and properties. Biol. Chem. Hoppe-Seyler 370, 217–227.

Ciccarelli, E., Vilardaga, J.-P., De Neef, P., Di Paolo, E., Waelbroeck, M., Bollen, A., Robberecht, P., 1994. Properties of the VIP-PACAP type II receptor stably expressed in CHO cells. Regul. Pept. 54, 397–407.

Couvineau, A., Rouyer-Fessard, C., Darmoul, D., Maoret, J.-J., Carrero, I., Ogier-Denis, E., Laburthe, M., 1994. Human intestinal VIP receptor: cloning and functional expression of two cDNA encoding proteins with different N-terminal domains. Biochem. Biophys. Res. Commun. 200, 769–774.

Gourlet, P., Vandermeers, A., Vandermeers-Piret, M.-C., Rathé, J., De Neef, P., Robberecht, P., 1996a. C-terminally shortened pituitary adenylate cyclase-activating peptides (PACAP) discriminate PACAP I, PACAP II-VIP_1 and PACAP II-VIP_2 recombinant receptors. Regul. Pept. 62, 125–130.

Gourlet, P., Vandermeers, A., Vandermeers-Piret, M.-C., De Neef, P., Robberecht, P., 1996b. Addition of the (28–38) peptide sequence of PACAP to the VIP sequence modifies peptide selectivity and efficacy. Int. J. Pept. Protein Res. 48, 391–396.

Gourlet, P., Vertongen, P., Vandermeers, A., Vandermeers-Piret, M.-C., Rathé, J., De Neef, P., Robberecht, P., 1997a. The long-acting vasoactive intestinal polypeptide agonist RO 25-1553 is highly selective of the VIP_2 receptor subclass. Peptides 18, 403–408.

Gourlet, P., Vandermeers, A., Vertongen, P., Rathé, J., De Neef, P., Cnudde, J., Waelbroeck, M., Robberecht, P., 1997b. Development of high affinity selective VIP_1 receptor agonists. Peptides 18, 1539–1545.

Gourlet, P., De Neef, P., Cnudde, J., Waelbroeck, M., Robberecht, P., 1997c. In vitro properties of a high affinity selective antagonist of the VIP_1 receptor. Peptides 18, 1555–1560.

Gourlet, P., Vandermeers, A., Van Rampelbergh, J., De Neef, P., Cnudde, J., Waelbroeck, M., Robberecht, P., 1997d. VIP-, helodermin- and PACAP-related peptides discriminate rat and human VIP_1 and VIP_2 receptors. Ann. N.Y. Acad. Sci. (in press).

Inagaki, N., Yoshida, H., Mizuta, M., Mizuno, N., Fujii, Y., Gonoi, T., Miyazaki, J.-I., Seino, S., 1994. Cloning and functional characterization of a third pituitary adenylate cyclase-activating polypeptide receptor subtype expressed in insulin-secreting cells. Proc. Natl. Acad. Sci. U.S.A. 91, 2679–2683.

Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K., Nagata, S., 1992. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. Neuron 8, 811–819.

Lutz, E.M., Sheward, W.J., West, K.M., Morrow, J.A., Fink, G., Harmar, A.J., 1993. The VIP_2 receptor: molecular characterization of a cDNA encoding a novel receptor for vasoactive intestinal peptide. FEBS Lett. 334, 3–8.

O'Donnell, M., Garippa, R.J., O'Neill, N.C., Bolin, D.R., Cottrell, J.M., 1991. Structure-activity studies of vasoactive intestinal polypeptide. J. Biol. Chem. 266, 6389–6392.

O'Donnell, M., Garippa, R.J., Rinaldi, N., Selig, W.M., Simko, B., Renzetti, L., Tannu, S.A., Wasserman, M.A., Welton, A., Bolin, D.R., 1994a. RO 25-1553: a novel, long-acting vasoactive intestinal peptide agonist: I. In vitro and in vivo bronchodilator studies. J. Pharmacol. Exp. Ther. 270, 1282–1288.

O'Donnell, M., Garippa, R.J., Rinaldi, N., Selig, W.M., Tocker, J.E., Tannu, S.A., Wasserman, M.A., Welton, A., Bolin, D.R., 1994b. RO 25-1553: a novel, long-acting vasoactive intestinal peptide agonist: II. Effect on in vitro and in vivo models of pulmonary anaphylaxis. J. Pharmacol. Exp. Ther. 270, 1289–1294.

Robberecht, P., Tatemoto, K., Chatelain, P., Waelbroeck, M., Delhaye, M., Taton, G., De Neef, P., Camus, J.C., Heuse, D., Christophe, J., 1982. Effects of PHI on vasoactive intestinal peptide receptors and adenylate cyclase activity in lung membranes: a comparison in man, rat, mouse and guinea pig. Regul. Pept. 4, 241–250.

Robberecht, P., Coy, D.H., De Neef, P., Camus, J.-C., Cauvin, A., Waelbroeck, M., Christophe, J., 1987. [D-Phe^4]peptide histidine–isoleucinamide ([D-Phe^4]PHI), a highly selective vasoactive intestinal peptide (VIP) agonist, discriminates VIP-preferring from secretin-preferring receptors in rat pancreatic membranes. Eur. J. Biochem. 165, 243–249.

Robberecht, P., Waelbroeck, M., De Neef, P., Camus, J.C., Coy, D.H., Christophe, J., 1988. Pharmacological characterization of VIP receptors in human lung membranes. Peptides 9, 339–345.

Robberecht, P., De Neef, P., Gourlet, P., Cauvin, A., Coy, D.H., Christophe, J., 1989. Pharmacological characterization of the novel helodermin/VIP receptor present in human SUP-T1 lymphoma cell membranes. Regul. Pept. 26, 117–126.

Robberecht, P., Gourlet, P., Vertongen, P., Svoboda, M., 1996. Characterization of the VIP receptor from SUP-T1 lymphoblasts. Adv. Neuroimmunol. 6, 49–57.

Salomon, Y., Londos, C., Rodbell, M., 1974. A highly sensitive adenylate cyclase assay. Anal. Biochem. 58, 541–548.

Svoboda, M., Tastenoy, M., Van Rampelbergh, J., Goossens, J.-F., De Neef, P., Waelbroeck, M., Robberecht, P., 1994. Molecular cloning and functional characterization of a human VIP receptor from SUP-T1 lymphoblasts. Biochem. Biophys. Res. Commun. 205, 1617–1624.

Usdin, T.B., Bonner, T.I., Mezey, E., 1994. Two receptors for vasoactive intestinal polypeptide with similar specificity and complementary distributions. Endocrinology 135, 2662–2680.

Vertongen, P., Schiffmann, S.N., Gourlet, P., Robberecht, P., 1997. Autoradiographic visualization of the receptor subclasses for vasoactive intestinal polypeptide (VIP) in rat brain. Peptides 18, 1547–1554.