The vasoactive intestinal peptide receptor on intact human colonic adenocarcinoma cells (HT29-D4)

Evidence for its glycoprotein nature

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We have previously shown that the mono [125 I]iodinated vasoactive intestinal peptide (125 I-VIP) could be covalently cross-linked on intact colonic adenocarcinoma cells (HT29). A major M_r 67000 and a minor M_r 120000 cross-linked polypeptides have been characterized [Muller, Luis, Fantini, Abadie, Giannellini, Marvaldi & Pichon (1985) *Eur. J. Biochem.* **151**, 411–417]. The glycoprotein nature of these species was investigated using endo- β -acetylglucosaminidase F (Endo F) treatment, enzymic and chemical desialylation and wheat germ agglutinin (WGA)–Sepharose affinity chromatography. Affinity-labelled VIP-binding proteins solubilized by Nonidet P-40 bound to WGA–Sepharose and could be eluted specifically with *N*-acetyl-D-glucosamine. Treatment with Endo F resulted in an increased electrophoretic mobility of both polypeptides. The major and the minor VIP-binding proteins were converted respectively into M_r 47000 and 100000 species, indicating removal of 20 kDa of *N*-linked oligosaccharides. Deglycosylation with trifluoromethanesulphonic acid also led to a 20 kDa loss in mass of the M_r 67000 component, indicating the absence of additional *O*-linked sugars on this polypeptide. The presence of sialic acid on the major VIP-binding protein was demonstrated after treatment of intact cells with neuraminidase or by chemical desialylation with hydrochloric acid. We conclude from this study that the VIP receptor from intact HT29-D4 cells is a glycoprotein with *N*-linked oligosaccharide side chains containing sialic acid.

INTRODUCTION

Vasoactive intestinal peptide (VIP) exhibits the general properties of a neurotransmitter with multiple functions (Costa *et al.*, 1980; Rosselin *et al.*, 1982). VIP is very abundant in gastrointestinal tract (Dimaline & Dockray, 1979) where it stimulates the secretion of water and electrolytes. After interaction with its membrane receptor, VIP induces a very large increase in the intracellular cyclic AMP level (Laburthe *et al.*, 1978) with subsequent activation of both cyclic AMP-dependent protein kinases and cyclic AMP phosphodiesterases (Mangeat *et al.*, 1981). The availability of affinity-labelling techniques using bifunctional reagents has enabled several groups to characterize different major polypeptides that were believed to be the VIP-binding components of the receptor. A major polypeptide of M_r 64000 was characterized on intact human adenocarcinoma cells (Muller *et al.*, 1985a) or on crude membranes of human normal colonic epithelial cells (Couvineau & Laburthe, 1985a). On rat epithelial intestinal cells a major polypeptide of M_r 73000 (high affinity binding site) was identified (Laburthe *et al.*, 1984), while on rat liver membranes a major polypeptide of M_r 48000 (Couvineau & Laburthe, 1985b) or M_r 53000 (Nguyen *et al.*, 1986) was obtained. Finally on intact human cells of lymphatic origin (MOLT 4b), a single polypeptide of M_r 47000 was characterized (Wood &

O'Dorisio, 1985). In spite of the increasing attention given to the interaction of VIP with specific receptors as well as VIP receptor-mediated endocytosis (Boissard *et al.*, 1984; Muller *et al.*, 1985b; Luis *et al.*, 1986), relatively little information about the fine structure of these receptors is known. It is only recently that Nguyen *et al.* (1986) have reported information on the glycoprotein nature of VIP receptor in plasma membranes isolated from rat liver. Nonetheless, very little is known about the carbohydrate content of this protein receptor that could explain, at least in part, the heterogeneity in molecular sizes in different systems.

Using intact HT29-D4 cells, a clone of HT29 cells (Fantini *et al.*, 1986), we now report that the major affinity-labelled VIP-binding protein on intact cells is a glycoprotein devoid of *O*-linked sugars but containing *N*-linked oligosaccharides with terminal sialic acid. We used as criteria specific binding to immobilized WGA and alteration in molecular mass after enzymic or chemical deglycosylation using either Endo F, neuraminidase, TFMS or chlorhydric acid.

EXPERIMENTAL

Materials

Porcine VIP was provided by Professor V. Mutt (Karolinska Institute, Stockholm, Sweden). 125 I-VIP was

Abbreviations used: VIP, vasoactive intestinal peptide; NP-40, Nonidet P-40; DSS, disuccinimidyl suberate; TFMS, trifluoromethanesulphonic acid; Con A, concanavalin A; WGA, wheat germ agglutinin; Endo F, endo- β -acetylglucosaminidase F; SDS/PAGE, sodium dodecyl sulphate/polyacrylamide-gel electrophoresis; PBS, phosphate-buffered saline.

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prepared by the chloramine-T method (Hunter & Greenwood, 1962), at a specific radioactivity of 250–300 Ci/g. *Vibrio cholerae* neuraminidase (1 unit/ml), methyl α -D-mannopyranoside and *N*-acetylglucosamine were obtained from Calbiochem (La Jolla, CA, U.S.A.). Con A–Sephadex and WGA–Sephadex (8 and 5 mg of lectin/ml of gel respectively) and *N*-acetylneuraminic acid (sialic acid) were purchased from Sigma. Endo F was a generous gift from Drs. J. Elder and S. Alexander (Research Institute of Scripps Clinic, La Jolla, CA, U.S.A.). TFMS and anisole were from Fluka (Buchs, Switzerland). DSS was obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.), and NP-40 was from BDH. Electrophoretic chemicals and M_r standards were from Bio-Rad. Dulbecco's modified Eagle's medium was from Gibco (Cergy Pontoise, France) and fetal calf serum was from Eurobio (Paris, France). Other chemicals were of highest purity commercially available.

Cell culture

The human colonic adenocarcinoma cell line HT29 (Fogh & Trempe, 1975) was obtained from Dr. Zweibaum (Paris, France) and cloned in our laboratory (Fantini *et al.*, 1986). HT29-D4 clone was routinely cultured in Dulbecco's modified Eagle's medium containing 4.5 g of glucose/l and 10% (v/v) fetal calf serum in a humidified atmosphere of air/CO₂ (19:1). Subcultures were obtained by harvesting exponentially growing cells with 0.53 mM-EDTA containing 0.05% trypsin in Dulbecco phosphate-buffered saline (PBS). Cells were seeded in 25 cm² or in 75 cm² culture flasks as indicated in each procedure.

¹²⁵I-VIP binding and affinity-labelling procedures

VIP binding reaction was performed according to a previously described method (Muller *et al.*, 1985a). Affinity-labelling of intact cells was obtained by cross-linking ¹²⁵I-VIP to HT29-D4 as described by Muller *et al.* (1985a) using the non-cleavable cross-linking reagent DSS instead of the cleavable dithio-bis(succinimidyl propionate). Briefly, after incubation with ¹²⁵I-VIP, HT29-D4 cell monolayers were washed to remove unbound polypeptide and treated with 2 mM-DSS in 60 mM-Hepes, pH 8.5, containing 0.16 M-NaCl. The cross-linking reaction was performed at 37 °C for 30 min. Before solubilization, cells from 75 cm² flasks were harvested by scraping with a rubber policeman and washed three times by centrifugation (600 g, 5 min, 4 °C) with ice-cold PBS containing a mixture of proteinase inhibitors: 150 μ M-phenylmethanesulphonyl fluoride, 2 mM-benzamide and 5 μ g of leupeptin/ml.

Detergent solubilization and lectin affinity chromatography

Detergent solubilization. Affinity-labelled cells were incubated for 2 h at 4 °C either in 50 mM-Hepes, pH 7.5, containing 0.16 M-NaCl, 1 mM-MgCl₂, 1 mM-CaCl₂ and proteinase inhibitors as above (WGA buffer) or in 50 mM-Tris/HCl, pH 7.5, containing 0.16 M-NaCl, 1 mM-CaCl₂, 0.5 mM-MnCl₂ and proteinase inhibitors (Con A buffer), both containing 1% NP-40. Insoluble material was removed by centrifugation at 15000 g for 20 min.

Lectin affinity chromatography. Columns were prepared in 1 ml single-use syringes filled with Sepharose-

coupled lectin. Before use, the gel was extensively washed (100 ml) with the WGA or Con A equilibration buffer (WGA or Con A buffer containing 0.1% NP-40). The soluble fraction was applied to the column and the appropriate equilibration buffer was run through the column until the radioactivity returned to the base-line. Equilibration buffer containing 0.5 M of complementary sugar (α -methyl mannoside for Con A or *N*-acetylglucosamine for WGA) was then added and column flow was stopped for 30 min. Fractions (0.2 ml) were then collected and monitored for their radioactivity in a γ -counter. Flow-through fractions and specifically eluted fractions were pooled separately and precipitated with 10% (w/v) trichloroacetic acid with 100 μ g of cytochrome *c* as carrier. Precipitated material was washed twice with ice-cold acetone (80% v/v in water), solubilized in SDS sample buffer (Laemmli, 1970) and analysed by SDS/PAGE. All these procedures were carried out at room temperature.

Deglycosylation procedures

Endo F treatment. The VIP receptor from affinity-labelled cells was solubilized in 0.1 M-sodium phosphate buffer, pH 6.1, containing 5 mM-EDTA, 1% 2-mercaptoethanol and 0.1% SDS, boiled for 2 min and allowed to cool at room temperature. The NP-40 concentration was then raised to 1%. This solution was divided into 100 μ l portions and 10 μ l of Endo F [provided in a 50% (v/v) glycerol/25 mM-EDTA solution] was added. Incubation was carried out as described by Elder & Alexander (1982). Each sample was analysed by SDS/PAGE after addition of an equal volume of SDS sample buffer.

Deglycosylation with TFMS. We used the method described by Edge *et al.* (1981) with some minor modifications. Affinity-labelled cells were solubilized under the same conditions as for Endo F treatment and the solution was desalted through a Sephadex G-50 column to exchange the buffer to 10 mM-Tris/HCl, pH 6.8, containing 0.1% NP-40. The desalted sample was lyophilized overnight, resuspended in 200 μ l of TFMS/anisole (2:1, v/v) and flushed with N₂. The tube was sealed and incubated first for 2 h at 0 °C and then for 2 h at –20 °C. The reaction was stopped by adding 500 μ l of 10 mM-triethanolamine followed by 100 μ l of 1 M-triethanolamine. The sample was then recovered by precipitation with 10% (w/v) trichloroacetic acid and analysed by SDS/PAGE.

Enzymic and chemical desialylation

Neuraminidase treatment. Cells grown in 25 cm² culture flasks were washed three times with PBS, pH 7.4, and incubated for 30 min at 37 °C in PBS adjusted to pH 5.5 with 0.1 M-H₃PO₄ containing 1% bovine serum albumin, 1 mg of bacitracin/ml, 25 mM-glucose, 4 mM-CaCl₂ and 50 m-units of neuraminidase/ml. After neuraminidase treatment, ¹²⁵I-VIP binding and cross-linking reactions were performed as described above. Monolayers were then solubilized in SDS sample buffer and analysed by SDS/PAGE.

Chemical desialylation. Cross-linked soluble receptor was obtained as previously described for Endo F treatment. The solution was desalted and lyophilized under the same conditions described for TFMS treatment except that the Sephadex G-50 column was equilibrated

in deionized water containing 0.1% NP-40. The lyophilization residue was resuspended either in water (control) or in 0.01M-HCl and incubated at 80 °C (Schauer, 1978). After 60 min of incubation, suspensions were cooled, proteins were recovered by precipitation with 10% (w/v) trichloroacetic acid and analysed by SDS/PAGE.

For determination of the total content of sialic acid, HT29-D4 cells were directly incubated in the presence of 0.01 M-HCl for 60 min at 80 °C.

Sialic acid determination. The amount of sialic acid recovered in the supernatant after neuraminidase treatment and after chemical desialylation of the lyophilization residue or of intact cells was determined after purification on Dowex 1 (Codington *et al.*, 1976) by the method of Warren (1959).

Gel electrophoresis, autoradiography and protein determination

Intact affinity-labelled cells and protein precipitates were solubilized in SDS sample buffer and run on a 12.5% acrylamide slab gel under reducing conditions, according to Laemmli (1970). Calibration proteins were: myosin (M_r 200000), β -galactosidase (M_r 116250), phosphorylase *b* (M_r 92000), bovine serum albumin (M_r 66200), ovalbumin (M_r 45000), carbonic anhydrase (M_r 31000), soybean trypsin inhibitor (M_r 21500) and lysozyme (M_r 14400). After electrophoresis, gels were fixed and stained with Coomassie Brilliant Blue R250. Dried gels were exposed to Kodak X-OMAT film for 7–21 days.

Protein concentration was determined according to Lowry *et al.* (1951).

RESULTS

Chromatography of VIP-binding proteins on lectin columns

Using HT29-D4 cells grown as a monolayer we have demonstrated, by affinity labelling with ^{125}I -VIP, that the cross-linked polypeptides were identical to those identified on non-cloned HT29 cells, as already described (Muller *et al.*, 1985a) (results not shown). To determine whether the VIP-binding proteins identified on intact HT29-D4 cells were glycoproteins, we initially chose to test their ability to be retained on immobilized WGA or Con A lectins. To this end, the affinity-labelled receptor was solubilized in the appropriate buffer and applied to the corresponding lectin. Con A- and WGA-Sepharose retained 5% and 43% of the applied radioactivity, respectively. When the equilibration buffer containing 0.5 M-*N*-acetylglucosamine was run through the WGA-Sepharose column, all the radioactivity adsorbed on the lectin was eluted (Fig. 1a). Only a very small amount of radioactivity bound to Con A-Sepharose could be recovered following elution with 0.5 M- α -methyl mannoside even when we used a mixture of 0.5 M- α -methyl mannoside/ethylene glycol (1:1, v/v) as recommended by Davey *et al.* (1976) (results not shown). The fractions containing radioactive material eluted from WGA-Sepharose were pooled, precipitated with 10% trichloroacetic acid and submitted to SDS/PAGE. Autoradiographic analysis showed that material eluted from the WGA column contained the M_r 67000 and M_r 120000 polypeptides previously characterized in intact HT29 cells. Additional components with higher molecular masses were also detected, probably due to aggregated material (Fig. 1b). We have been unable to detect any

Fig. 1. Affinity chromatography of VIP-binding proteins on WGA-Sepharose

Conditions are as described in the Experimental section. (a) Bound material was eluted with 0.5 M-*N*-acetylglucosamine (GlcNAc) in WGA buffer containing 0.1% NP-40. (b) The eluted material was precipitated with trichloroacetic acid and analysed by SDS/PAGE and autoradiography. Migration of M_r standards is indicated on the right.

cross-linked polypeptides in the flow-through fractions after SDS/PAGE analysis (results not shown).

Deglycosylation of VIP-binding proteins

To analyse further the glycoprotein nature of the VIP-binding complex, we treated the solubilized cross-linked material with Endo F. This enzyme hydrolyses the glycosidic bond of the *NN'*-diacetylchitobiose core, next to the asparagine (Elder & Alexander, 1982). Autoradiographic analysis showed that treatment with Endo F for 1 h resulted in a decreased electrophoretic mobility of the major M_r 67000 and minor M_r 120000 polypeptides. The two species were converted into M_r 61000 and M_r 113000 components respectively (Fig. 2a, lane B). Treatment for 4 h with the enzyme resulted in the complete conversion of the major M_r 67000 polypeptide into a M_r 47000 component (Fig. 2b, lane B). The minor M_r 120000 polypeptide was converted to a molecular form migrating with an apparent M_r of 100000. When treatment with Endo F was performed for as long as 12 h, no change in electrophoretic mobility of the M_r 47000 and M_r 100000 species was observed (Fig. 2b, lane

C). In control experiments (i.e. without Endo F) no change in the electrophoretic mobility of the M_r 67000 polypeptide was observed after incubation under the same conditions for 1 h (Fig. 2a, lane A) or 12 h (Fig. 2b, lane A).

Assuming that the major M_r 47000 cleavage product shown in Fig. 2(b) (lanes B and C) represents an affinity-labelled protein devoid of *N*-linked oligosaccharides, it was of interest to investigate the additional presence of *O*-linked sugars on this polypeptide. To this end, TFMS was used. This strong organic acid removes all oligosaccharide side chains from proteins by hydrolysis of glycosidic intrachain bonds as well as bonds between serine (or threonine) and the first sugar of the *O*-linked chain. The asparagine-*N*-acetylglucosamine bond of *N*-linked sugars is only partially cleaved (Edge *et al.*, 1981). As shown in Fig. 3, the major reaction product has the same apparent M_r (47000) as the one obtained after Endo F treatment. The minor M_r 120000 polypeptide was not visible under these experimental conditions. The result of this experiment indicates that no additional *O*-linked oligosaccharides are present on the VIP-binding M_r 67000 protein. The very drastic treatment with TFMS led to a loss of cross-linked material compared with the control.

Removal of sialic acid

Since WGA specifically binds to glycoproteins containing sialic acid and *N*-acetylglucosamine (Bhavanandan & Katlic, 1979) and because most complex oligosaccharides contain terminal sialic acid, we tested the VIP receptor for its sensitivity to neuraminidase and

Fig. 2. Endo F treatment of VIP-binding proteins

Conditions are as described in the Experimental section. (a) Solubilized affinity-labelled material was incubated in the absence (A) or presence (B) of Endo F at 37 °C for 1 h. (b) Solubilized material was treated for 4 h (B) or 12 h (C) with Endo F or for 12 h without the enzyme (A).

Fig. 3. Deglycosylation of VIP-binding proteins with TFMS

Conditions are as described in the Experimental section. Lyophilized material was incubated in the absence (A) or presence (B) of TFMS.

chemical desialylation. Fig. 4(a) (lane B) shows that when intact cells were treated with neuraminidase before performing VIP binding and cross-linking reactions, the electrophoretic mobility of the M_r 67000 species was slightly increased. Treatment with 0.01 M-HCl at 80 °C for 60 min of lyophilized soluble material (see the Experimental section) resulted in a 3 kDa decrease in the mass of the major VIP-binding protein (Fig. 4b, lane B), corresponding to approx. 10 sialic acid residues/polypeptide. Results of a quantitative sialic acid determination on HT29-D4 cells are listed in Table 1; 32% or the total sialic acid was released after neuraminidase treatment and 69% after chemical desialylation.

Fig. 4. Removal of sialic acid from VIP-binding proteins

Conditions are as described in the Experimental section. (a) Cell monolayers were incubated in the absence (A) or presence (B) of neuraminidase followed by 125 I-VIP binding, cross-linking reaction and SDS/PAGE. (b) Lyophilized material was resuspended in water (A) or in 0.01 M-HCl (B) and incubated at 80 °C for 1 h. Proteins were then precipitated with trichloroacetic acid and analysed by SDS/PAGE.

Table 1. Quantification of sialic acid released after chemical or enzymic treatment

Each value represents the average of triplicate analysis of two separate experiments, with S.E.M. less than 10%. 'Total sialic acid' is that chemically released from whole cells by HCl treatment as described in the Experimental section.

	Sialic acid	
	(nmol/mg of protein)	(% of total)
Total sialic acid	1.40	100
Neuraminidase-released sialic acid from intact cells	0.45	32
Chemically-released sialic acid from solubilized material	0.96	69

DISCUSSION

In this study, we used the bifunctional reagent DSS to cross-link 125 I-labelled VIP to its binding site on intact HT29-D4 cells. Two polypeptides of M_r 67000 (major) and M_r 120000 (minor) were characterized. In our previous paper (Muller *et al.*, 1985a) we have demonstrated, using the cross-linker reagent dithio-bis(succinimidyl propionate) and non-cloned HT29 cells, that the major affinity-labelled polypeptide (M_r 67000) has the properties of a high-affinity VIP-binding site.

We now report that the major M_r 67000 and the minor M_r 120000 polypeptides are glycoproteins containing *N*-linked glycans accounting for 20 kDa. This conclusion was based on the following criteria: (1) the solubilized affinity-labelled polypeptides were retained on a WGA-Sepharose column and specifically eluted by *N*-acetylglucosamine; (2) Endo F treatment of the solubilized affinity-labelled receptor converted the major M_r 67000 and the minor M_r 120000 polypeptides into M_r 47000 and M_r 100000 components respectively. These data strongly suggest that the minor M_r 120000 polypeptide is not a dimer of the M_r 67000 component.

The increased electrophoretic mobility we have observed after Endo F treatment was not due to proteolysis, but to deglycosylation of the affinity-labelled polypeptides. This was demonstrated by the perfect stability of the labelled species even after 12 h incubation in the Endo F buffer lacking the enzyme.

Additional data concerning the glycoprotein nature of the major M_r 67000 polypeptide are as follows.

(1) Chemical treatment of solubilized affinity-labelled material with the organic acid TFMS generated a polypeptide of M_r 47000, probably identical with the one obtained after Endo F treatment. These data demonstrate that the M_r 67000 species (a M_r 64000 polypeptide, assuming 1 molecule of 125 I-VIP/polypeptide) is an *N*-linked glycoprotein devoid of *O*-linked sugars.

(2) The presence of sialic acid on the M_r 67000 polypeptide was demonstrated by neuraminidase treatment of HT29-D4 cells or chemical desialylation of the solubilized affinity-labelled receptor. We observed an increased electrophoretic mobility of the M_r 67000 polypeptide which corresponds to a loss in molecular mass of 3 kDa; this could account for approx. 10 sialic acid residues/polypeptide. It is worth noting that HT29-D4 cells appear to be very poor in sialic acid content (Table 1) compared with other human transformed cells from liver or pancreas (Mabry & Carubelli, 1972) or with human melanoma cells (Reynier *et al.*, 1984). Furthermore most of the HT29-D4 cell surface proteins bind weakly or not at all to WGA-Sepharose (results not shown), probably due to the lack of terminal sialic acid. Hence sialoglycoproteins, to which the M_r 67000 polypeptide belongs, seem to represent a minority among the HT29-D4 cell surface glycoproteins.

A recent study concerning the VIP receptor from rat liver membranes demonstrated the glycoprotein nature of two affinity-labelled polypeptides of M_r 80000 and M_r 56000 (Nguyen *et al.*, 1986). These authors indicated: (1) that the loss in molecular mass observed for both polypeptides after Endo F treatment was 19 kDa, and (2) that the affinity-labelled M_r 56000 polypeptide was derived from the M_r 80000 component after removal of an M_r 24000 peptide fragment by proteolysis that could occur during the membrane preparation. According to

these results, the high-affinity binding site on rat liver membrane was the affinity-labelled M_r 80000 component, while for Couvineau & Laburthe (1985b) the high-affinity binding site from rat liver membrane was the affinity-labelled polypeptide of M_r 51000.

These contradictory data raised the question of chemical modifications (proteolysis or deglycosylation) following membrane preparation. For this reason, the data we reported here were obtained after cross-linking experiments performed on intact cells to eliminate these potential modifications. If we compare our previous data with those presented in the present paper, we can conclude that the same affinity-labelled polypeptides were found either after direct analysis of the cross-linked material by SDS/PAGE (Muller *et al.*, 1985a) or after solubilization in presence of NP-40, WGA affinity chromatography and subsequent electrophoresis analysis. This demonstrates a good stability of the cross-linked material under our experimental conditions. Yet it is important to note that, when membranes of HT29 cells were used instead of intact cells, cross-linking with ^{125}I -VIP resulted in a polypeptide of M_r 49000 (Marvaldi *et al.*, 1986) that could be a deglycosylated form of the VIP receptor. This deglycosylation could be due to the release of an endoglycosidase in the medium during membrane preparation. This point of course needs further investigation, particularly since Pierce *et al.* (1979) have described such endoglycosidase activity in rat liver cytosolic fraction.

Our results indicated that the affinity-labelled polypeptide of M_r 67000 was totally converted into a single smaller polypeptide of M_r 47000 after 4 or 12 h exposure to Endo F. The loss in molecular mass of 20 kDa observed after deglycosylation was in good agreement with the data of Nguyen *et al.* (1986) for the VIP receptor on liver plasma membrane. It is interesting to note that the affinity-labelled deglycosylated polypeptide (M_r 47000) found in HT29-D4 cells had an M_r similar to that of the deglycosylated polypeptide found by Nguyen *et al.* (1986) after gel filtration chromatography of solubilized VIP receptor from rat liver plasma membrane.

The discrepancies in the data concerning the VIP receptor from different tissues must be clarified further, but they could be explained by chemical modifications

due to membrane preparation or to cellular heterogeneity, particularly for liver.

From another point of view, our data underline the striking structural homology between the VIP receptor from human HT29-D4 cells and the glucagon receptor from rat liver membranes (Iyengar & Herberg, 1984; Herberg *et al.*, 1984), as shown in Table 2. It is tempting to raise the hypothesis that glucagon and VIP receptor belong to the same family of receptor proteins, as already proposed by Laburthe (1985).

We thank Drs. John H. Elder and Stephen Alexander for the generous gift of Endo F, and Mme Hermine Bazin and Dr. Michel Hirn for their help in TFMS experiments. We also thank Ms. Jeanine Secchi, Messrs Fernand Giannellini and Roger Soirat for their expert technical assistance. This work was supported in part by the Centre National de la Recherche Scientifique (CNRS, UA no. 202), by the Institut National de la Santé et de la Recherche Médicale (INSERM, grant CRE 847006) and by the Association pour le Développement de la Recherche sur le Cancer (ARC, grant 6187).

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Table 2. Structural homology between VIP-binding and glucagon-binding proteins

	M_r of the binding protein–ligand complexes for:	
	Glucagon receptor (rat liver membrane)	VIP receptor (intact HT29 cells)
Minor component	113000 ^a	120000 ^c
Major component	63000 ^b	67000 ^c
Minor component	33000 ^b	34000 ^c
Deglycosylated form of the major component	45000 ^b	47000 ^a

^a Herberg *et al.* (1984).

^b Iyengar & Herberg (1984).

^c Muller *et al.* (1985a).

^d This paper.

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Received 5 August 1986/ 6 October 1986; accepted 23 October 1986