Peptide Agonist Docking in the N-terminal Ectodomain of a Class II G Protein-coupled Receptor, the VPAC1 Receptor

PHOTOAFFINITY, NMR, AND MOLECULAR MODELING

Received for publication, December 14, 2005; in revised form, February 13, 2006. Published, JBC Papers in Press, March 6, 2006, DOI 10.1074/jbc.M513305200

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The neuropeptide vasoactive intestinal peptide (VIP) strongly impacts on human pathophysiology and does so through interaction with class II G protein-coupled receptors named VIP putitary adenylate cyclase-activating peptide (PACAP) receptors (VPACs). The molecular nature of VIP binding to receptors remains elusive. In this work, we have docked VIP in the human VPAC1 receptor by the following approach. (i) VIP probes containing photolabile residues in positions 6, 22, and 24 of VIP were used to photolabel the receptor. After receptor cleavage and Edman sequencing of labeled receptor fragments, it was shown that Phe6, Tyr22, and Asn24 of VIP are in contact with Asp107, Gly116, and Cys122 in the N-terminal ectodomain (N-ted) of the receptor, respectively. (ii) The structure of VIP was determined by NMR showing a central α-helix, a disordered N-terminal His1–Phe6 segment and a 310 Ser25–Asn28 helix termination. (iii) A threedimensional model of the N-ted of hVPAC1 was constructed by using the NMR structure of the N-ted of the hAVP receptor and the central片段vitamin C receptor–releasing factor receptor 2β as a template. As expected, the fold is identified as a short consensus repeat with two antiparallel β sheets and is stabilized by three disulfide bonds. (iv) Taking into account the constraints provided by photoaffinity, VIP was docked into the hVPAC1 receptor N-ted. The 6–28 fragment of VIP nicely lies in the N-ted C-terminal part, but the N terminus region of VIP is free for interacting with the receptor transmembrane region. The data provide a structural rationale to the proposed two–step activation mechanism of VPAC receptor and more generally of class II G protein-coupled receptors.

The class II of G protein-coupled receptors (GPCRs)3 comprises receptors for a series of large peptide hormones (>27 amino acid residues) that clearly impact on human pathophysiology such as glucagon, glucagon-like peptides, secretin, vasoactive intestinal peptide (VIP), corticotropin-releasing factor (CRF), growth hormone releasing factor, parathyroid hormone, or calcitonin (1–4). Like class I receptors, they have a seven-transmembrane domain core, although little sequence homology is found in the transmembrane helices between the two classes of GPCRs (1–4). Unlike most class I receptors, they have a large N-terminal ectodomain (N-ted), which is crucial for peptide hormone agonist binding (1–4). The class II receptor ectodomains are of similar length and contain a few highly conserved residues including 6 cysteine residues that provide required secondary structure of the domain (1–4).

The GPCR field has benefited greatly from atomic resolution of the class I rhodopsin receptor (5). Since rhodopsin has a very small N-ted, the determination of its structure provided no clue to understand the process of peptide hormone binding to class II receptors. Experimentally based molecular modeling of the N-ted has been reported for the hPTH1 receptor (6), and a structural model has been constructed for the hVPAC1 by sequence homology with a functionally unrelated protein, the structure of which was available in the protein data bank (7). However, the real breakthrough in the field came recently when Grace et al. (8) determined the NMR structure of the N-ted of the CRF 2β receptor.

The VPAC1 receptor for the neuropeptides VIP and pituitary adenylate cyclase-activating peptide (PACAP) has been extensively studied for many years by site-directed mutagenesis and molecular chimerism and is prototypic of class II GPCRs (reviewed in Ref. 9). It appeared that the N-ted of the VPAC1 receptor plays a crucial role for VIP binding, although it is not sufficient to ensure high affinity (9). The physical sites of interaction between VIP and its receptor had remained elusive until recent photoaffinity experiments showing that the side chains of positions 6 and 22 of VIP are in direct contact with the N-ted of the receptor (10, 11). Since it is well known that VIP has diffuse pharmacophoric domains, with the amino acid residues important for biological activity being distributed along the whole 28-amino acid peptide chain (12), incorporation of a photoactivable group on the side chain at other positions of VIP is clearly needed. Moreover, contacts between residues in VIP and its receptor should be determined to dock VIP in its binding site.

In this context, the present work has several complementary goals: (i) to develop a new photoaffinity probe by substituting benzoyl–benzoyl–L-Lys for asparagine 24 and to determine spatial approximation between the hVPAC1 receptor and the side chain of position 24 of VIP; (ii) to...
determine by radiochemical Edman degradation the pairs of residues in VIP and in the receptor that ensure the covalent attachment of the above mentioned $^{125}$I-[Bz$_2$-K$_{24}$]-VIP probe but also of two photoaffinity probes previously described i.e. $^{125}$I-[Bpa$_8$]-VIP (11) and $^{125}$I-[Bpa$_{28}$]-VIP (10); (iii) to determine the NMR structure of VIP, which has not been totally reported yet (13), although NMR data are available for some VIP fragments or analogues (14); (iv) to develop a new three-dimensional molecular model of the N-ted of the VPAC1 receptor by homology modeling using the equivalent domain in the CRF 2β receptor (8) as a template; and (v) to dock VIP in the N-ted of the receptor by using constraints provided by photoaffinity labeling, NMR structure of VIP, and molecular model of the N-ted of the hVPAC1 receptor. The present study describes the data and provides the first peptide agonist docking for a class II GPCR.

EXPERIMENTAL PROCEDURES

**Materials**—The hVPAC1 receptor was cloned in our laboratory (15). VPAC1 receptor was fused in the C-terminal position with green fluorescent protein (16) and used in all studies. This receptor construct displays the same phenotype as the wild-type receptor in terms of VIP binding and activation of adenylyl cyclase (16). All constructs (wild type and mutants) were stably transfected in CHO cells as described (12). The CHO-F7 clone expressing the hVPAC1 construct was used in most experiments. Cell membranes were obtained exactly as described (12). Native VIP and photolabile VIP probes [Ala$_{17}$, para-benzoyl-l-Phe$_8$]-VIP-28, [Ala$_{17}$, para-benzoyl-l-Phe$_{22}$]-VIP-28, and [Ala$_{17}$, benzoylbenzoyl-l-Lys$_{29}$]-VIP-28 were obtained by custom synthesis from NeoSys (Strasbourg, France) and were referred to as [Bpa$_8$]-VIP (11), [Bpa$_{28}$]-VIP (10), and [Bz$_2$-K$_{24}$]-VIP, respectively. $^{125}$I-VIP and $^{125}$I-photolabile VIP probes were prepared and purified as described (12). hVPAC1 receptor mutants that incorporated additional sites for CNBr cleavage in positions 104, 108, 113, 114, and 120 were obtained by methionine substitution as reported (17).

Radioreceptorassay and Adenylyl Cyclase Activity Measurement—Ligand binding to membrane preparations was carried out as described (12) with 0.05 nM $^{125}$I-VIP in the presence of increasing concentrations of VIP or [Bz$_2$-K$_{24}$]-VIP. The concentration of peptides that elicited half-maximal inhibition of specific $^{125}$I-VIP binding ($K_i$) was determined by computer analysis. Adenylyl cyclase activity in cell membranes was assayed in the presence of increasing concentrations of VIP or [Bz$_2$-K$_{24}$]-VIP as described (12). Dose-response curves were fitted, and concentrations of peptides giving half-maximal responses (EC$_{50}$) were calculated using the Prism software suite.

Receptor Photoaffinity Labeling—Transfected CHO cells were incubated in darkness with 10 nM $^{125}$I-[Bpa$_8$]-VIP, $^{125}$I-[Bpa$_{28}$]-VIP, or $^{125}$I-[Bz$_2$-K$_{24}$]-VIP in 20 mM HEPEs buffer, pH 7.4, as described (10, 11). After a 1-h incubation, cells were washed and pelleted by centrifugation, and the pellets were resuspended in 4 ml of 20 mM HEPEs buffer containing 1 mM phenylmethylsulfonyl fluoride and 0.1 mM tosyl-L-lysine chloromethyl ketone. Cell suspensions were photolyzed ($\lambda = 365$ nm) as described (10) on ice at the distance of 2 cm. After 30 min of UV exposure, cells were collected and washed two times (10). The photolabeled receptors were then analyzed directly by electrophoresis or after chemical or enzymatic cleavage.

Photoaffinity-labeled Receptor Cleavage—Cleavage of labeled hVPAC1 receptor or mutants with CNBr, peptide N-glycosidase F, or endoproteinase Glu-C was performed as described (10, 11). Products of cleavage were resolved on NuPAGE 4–12% Bis-Tris gel using MES SDS running buffer system from Invitrogen under reducing conditions in the presence of 20 mM dithiothreitol (10, 11). After electrophoresis, gels were dried and exposed to x-ray film as described (10). The apparent molecular masses of radiolabeled receptor fragments were determined by interpolation on a plot of the mobility of the Rainbow$^\text{TM}$ colored protein molecular weight markers from Amersham Biosciences or BenchMark$^\text{TM}$ prestained protein ladder from Invitrogen versus the log values of their masses.

Edman Degradation Sequencing—Identification of receptor fragments affinity-labeled with VIP probes was performed by Edman degradation sequencing (18). Briefly, cells expressing receptor mutants were incubated with labeled photoaffinity probes, washed, and photolyzed. Cells were then washed and collected by centrifugation, and pellets were analyzed by NuPAGE electrophoresis. The labeled receptor band was cut out from the gel, electroeluted, and lyophilized under vacuum, and then CNBr fragments were obtained as described (10, 11). CNBr fragments were then dissolved in a buffer containing 10 mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and immobilized in the C terminus position by coupling to 20 mg of N-(2-aminoethoxy)-1-3-aminopropyl glass beads for 2 h at room temperature. It should be noted that the carboxyl group of $^{125}$I-photolabile VIP probes was amidated as in the wild-type VIP and did not interfere with the coupling to the beads. Immobilized fragments were subjected to manual Edman degradation sequencing up to six cycles as follows. Beads were washed with 50 μl of triethylamine for three times and dried. 60 μl of triethylamine/methanol/phenylisothiocyanate mixture (1:7:1) were added to beads and incubated at 50 °C for 5 min. Beads were washed three times and dried. Dried beads were incubated with 50 μl of trifluoroacetic acid for 5 min at 25 °C and washed three times with 200 μl of methanol. Washing methanol effluents were pooled and evaporated, and the radioactivity released was quantified in a $\gamma$-spectrometer. The beads were dried and ready to use for the next cycle.

Molecular Modeling—The three-dimensional NMR structure of the amino-terminal domain of the mouse CRF 2β receptor (8) was used to model the N-ted of hVPAC1 receptor by homology. Sequence of the N-ted (1–137) of the hVPAC1 receptor was aligned with the N-terminal domain of CRF 2 receptor (Protein Data Bank code: 1U34) using FASTA algorithm (19). To further check the homology, the hydrophobic cluster analysis plots of both sequences were compared using the Visualfasta program (D. Durand, Paris, France). Based on sequence alignment, 100 homology models were built using Modeler version 6.0, and the objective function was calculated for each model to select the best score (7). Energy minimization calculation was performed with the AMBER forcefield. The model has 77% of the φ, ψ angle pairs in the allowed region of Ramachandran plot, indicating a correct stereochemistry.

### TABLE 1

| Statistics of 10-lowest energy structures of VIP |
|-----------------------------------------------|
| r.m.s.d., root mean square deviation.          |
| Number of distance restraints                  |
| Unambiguous                                     |
| 527                                            |
| Intraresidues                                   |
| 269                                            |
| Sequential                                     |
| 118                                            |
| Medium range                                   |
| 140                                            |
| Ambiguous                                      |
| 5                                              |
| r.m.s.d. to average structure, Å               |
| Backbone                                       |
| 1.80 ± 0.50                                    |
| Heavy atoms                                    |
| 2.43 ± 0.42                                    |
| Backbone (residues 7–24)                       |
| 0.74 ± 0.33                                    |
| Heavy atoms (residues 7–24)                    |
| 1.58 ± 0.35                                    |
| Ramachandran plot statistics, %               |
| Most favored region                            |
| 83.5                                           |
| Additionally allowed region                    |
| 13.8                                           |
| Generously allowed region                      |
| 1.9                                            |
| Diseallowed region                             |
| 0.8                                            |

**Class II GPCR Ectodomain**

| Material | Description |
|----------|-------------|
| 125I-VIP | Radiolabeled peptide for binding studies. |
| [Bpa$_8$]-VIP | VIP fragment with bromophenylalanine residues. |
| [Bpa$_{28}$]-VIP | VIP fragment with bromophenylalanine residues. |
| [Bz$_2$-K$_{24}$]-VIP | VIP fragment with benzoylalanine and benzoyllysine residues. |
TABLE 2

hVPAC1 receptor fragments and residues covalently attached to photoaffinity probes

| Photoaffinity probe     | Labeled receptor fragment | Ref. | Receptor residue involved in covalent bond | Ref.   |
|-------------------------|---------------------------|------|------------------------------------------|--------|
| 125I-[BpaF]-VIP         | 104–108                   | 11   | Asp^{107}                                | This paper |
| 125I-[Bpa^{23}]-VIP     | 109–120                   | 10   | Gly^{116}                                | This paper |
| 125I-[Bza-K^{24}]-VIP   | 121–133                   |      | Cys^{122}                                | This paper |

RESULTS AND DISCUSSION

Analysis of Spatial Approximation between VIP and Its Receptor by Photoaffinity—In previous studies, it was shown that photolabile residues in positions 6 and 22 of VIP covalently labeled receptor fragments 104–108 (11) and 109–120 (10) in the N-ted of the hVPAC1 receptor, respectively (Table 2). We further extend these studies by synthesizing [Bza-K^{24}]-VIP, which consists of substitution of Asn^{24} residue by the photolabile residue benzoylbenzoyl-l-Lys. This probe was equipotent with native VIP in inhibiting 125I-VIP binding (K_{i} of 0.6 nM) and in stimulating adenylyl cyclase activity (E_{max} of 0.3 nM) in CHO cells expressing the hVPAC1 receptor (Fig. 1). This is not surprising since previous alanine scanning studies showed that the amino acid residue in position 24 of VIP is not important for biological activity of the peptide (12). Incubation of 125I-[Bza-K^{24}]-VIP with transfected CHO cells followed by UV exposure and NuPAGE analysis of proteins revealed a single band at approximate M_{r} = 95,000, which disappeared when the labeled probe was incubated in the presence of 1 μM cold VIP (Fig. 2).

NMR Experiments and Structure Determination of VIP—VIP was dissolved at 2 mM in 90:10 H_{2}O:D_{2}O phosphate buffer (pH 6.0) containing 30% (v/v) trifluoroethanol-d_{3} as a helix stabilizer. 1H-NMR experiments were carried out on a DRX 600 Bruker spectrometer at 25 and 30 °C. Total correlated spectroscopy and NOE spectroscopy were recorded with mixing times of 80 and 150 ms, respectively. From the NOE spectroscopy spectra, a total of 527 NOEs were observed. The NOE intensities were used as input to ARIA1.2 (20). An ensemble of 100 structures was calculated with the ARIA default protocol. The 10 lowest energy conformers were minimized using CHARMM29 (21) and evaluated with the ARIA default protocol. The 10 lowest energy conformers of VIP and the selected three-dimensional model (43–137) of the N-ted of the receptor were carried out before and after the molecular dynamics run. A distance-dependent dielectric coefficient has been used to approximate the solvent screening effect without including explicit water molecules.
mentioned above, previous studies demonstrated that $^{125}$I-[Bpa$^6$]-VIP and $^{125}$I-[Bpa$^{22}$]-VIP photoaffinity probes covalently labeled receptor fragments 104–108 (11) and 109–120 (10) of the VPAC1 receptor, respectively (Table 2). We extended these studies by determining which individual residues of the receptor are actually concerned by the covalent attachment to the photolabile VIP probes. CHO cells expressing the VPAC1 receptor were incubated with $^{125}$I-[Bpa$^6$]-VIP, and CNBr was cleaved followed by digestion with endoproteinase Glu-C and trypsin as reported (11). This procedure resulted in the identification of a 2-kDa labeled fragment corresponding to the 104–108 sequence (not shown). This 2-kDa fragment was subjected to Edman degradation sequencing. A peak of radioactivity was observed in the fourth cycle of degradation (Fig. 3B), indicating that the $^{125}$I-[Bpa$^6$]-VIP probe was covalently attached to Asp$^{107}$ in the receptor (Table 2). Next, we determined which residue within the receptor is covalently to the $^{125}$I-[Bpa$^{22}$]-VIP probe. As shown previously, this probe is covalently attached to the 109–120 receptor fragment (Table 2). Direct Edman sequencing of this fragment did not work, most probably because the residue covalently bound to the probe is far from the N terminus of this rather long fragment. In this context, we developed new mutants in which some selected residues were substituted by methionine to introduce new CNBr cleavage sites e.g. E108M/E114M and L113M/I120M double mutants. These mutants were stably transfected in CHO cells and were shown to bind VIP with affinities identical to that of the wild-type receptor (not shown). Photoaffinity labeling of transfected cells with $^{125}$I-[Bpa$^{22}$]-VIP followed by CNBr treatment and electrophoresis showed labeled bands at ~6 and 4.5 kDa for the E108M/E114M and L113M/I120M hVPAC1 receptor mutants, respectively. These bands unambiguously corresponded to the labeling of the receptor fragments 115–137 and 114–120, respectively. They were submitted to Edman sequencing. A peak of radioactivity was observed in the second cycle for the 6-kDa band corresponding to the 115–137 fragment (Fig. 3C), indicating that $^{125}$I-[Bpa$^{22}$]-VIP is covalently bound to Gly$^{116}$. For the 4.5-kDa band corresponding to the 114–120 fragment, the peak of radioactivity was observed in the third cycle (Fig. 3D), also indicating that Gly$^{116}$ is the site of covalent attachment of the probe (Table 2). Altogether, the photoaffinity approach clearly indicates that the central part of VIP, at least between residues in positions 6 and 24, is in direct contact with the N-terminus of the hVPAC1 receptor within the short segment between Asp$^{107}$ and Cys$^{122}$.

**NMR Structure of VIP, Structural Model of the VPAC1 Receptor Ectodomain, and Docking of VIP**—To position VIP within the N-terminus of the receptor, we determined the structure of VIP by NMR and also...
developed a new molecular model of the receptor N-ted. The average NMR structure of VIP contains a central \( \alpha \) helix comprising residues 7–24 (Table 1, Fig. 4). Two side chain interactions are observed, Asn\(^7\)–Arg\(^{12}\) and Lys\(^{20}\)–Asn\(^{24}\), which may contribute to stabilize the helical conformation. The N-terminal His\(^1\)–Phe\(^6\) segment is largely disordered, and no persistent structural feature can be highlighted. In contrast, the flexibility of the C-terminal Ser\(^{25}\)–Asn\(^{28}\) segment appears to be more restricted, and its average conformation is close to a helical state. Moreover, the occurrence of a CO(Ser\(^{25}\))–NH(Asn\(^{28}\)) hydrogen bond may be assigned to a C-capping interaction consistent with a classical \( \beta \) helix termination. An asymmetrical distribution of the bulky hydrophobic side chains is observed in the Thr\(^7\)–Asn\(^{24}\) segment. This region contains two successive hydrophobic patches, Leu\(^{13}\) and Met\(^{17}\) and Val\(^{19}\) and Leu\(^{23}\), the locations of which are almost diametrically opposed with respect to the helix axis (Fig. 4).

Next, we developed a new three-dimensional molecular model of the N-ted of the hVPAC1 receptor domain. The latest breakthrough in studies of class II GPCRs was the determination of the NMR structure of the N-ted of the mouse CRF2 (mCRF2) receptor (8). A sequence alignment between the N-teds of hVPAC1 receptor (sequence 44–137) and mCRF2 receptor (sequence 39–133) was performed using FASTA algorithm (Fig. 5). This alignment revealed 22% identity and 42% homology between the N-ted of the two receptors. Alignment of hydrophobic clusters mostly supports homologous folds (Fig. 5). The quality of alignment is evaluated by the hydrophobic cluster analysis score, which is 45%. Based on the sequence alignment, a three-dimensional model of the N-ted of the hVPAC1 receptor (sequence 44–137) was constructed using Modeler 6.0 and refined by energy minimization (AMBER force field). The local root mean square distance between the hVPAC1-(44–137) three-dimensional model and the mCRF2-(39–133) NMR structure is 1.54 Å, indicating similar geometrical parameters of the two proteins. As expected, the structure contains two antiparallel \( \beta \) sheets and is stabilized by three disulfide bonds between residues Cys\(^{50}\)–Cys\(^{72}\), Cys\(^{63}\)–Cys\(^{105}\), and Cys\(^{86}\)–Cys\(^{122}\) by a putative salt bridge involving Asp\(^{68}\)–Arg\(^{103}\), sandwiched between the aromatic rings of Trp\(^{73}\) and Trp\(^{110}\) (Fig. 6). Quite interestingly, previous site-directed mutagenesis of the hVPAC1 receptor indicated that all these residues, which are likely to stabilize the polypeptide fold, are essential for receptor binding activity (reviewed in Ref. 9). Indeed, the individual mutation of all the above listed cysteine residues resulted in completely inactive VPAC1
receptor in terms of VIP binding and VIP-stimulated cAMP production (25). Similarly, the mutation into alanine of Asp68, Trp73, and Trp110, which are highly conserved in class II GPCRs, has been shown to inactivate the VPAC1 receptor (7). The same holds true for the mutation of Arg103 into alanine. Two other residues of hVPAC1 receptor, Pro74 and Gly109, which are highly conserved in class II receptor N-ted, have been shown previously to be essential for hVPAC1 receptor activity (26). Pro74 is presumably important to end the strand, and Gly109 may contribute to the folding of the interconnecting loop between β2 and β3 strands (Fig. 6). As previously noted for the CRF receptor β2, this fold is identified as a short consensus repeat also called complement protein modules and Sushi domains (27, 28). In general, where short consensus repeats occur toward the N terminus of cell surface receptors, they are involved in ligand recognition rather than in playing a pure structural role. This is very consistent with our photoaffinity data (see above) as well as the docking of VIP (see below). The conservation of short consensus repeats in class II G protein-coupled receptors for peptide hormones (8) supports the idea that this fold of the N-ted receptor also plays a crucial role for peptide agonist binding in this class of GPCRs. Finally, it is worth pointing out that the N-ted of the hVPAC1 receptor contains three N-glycosylation sites on Asn58, Asn69, and Asn100, which are occupied by a 9-kDa carbohydrate (24). It is worth noting that the location of these asparagine residues in this model (Fig. 6) allows good exposure of the carbohydrate moieties to the aqueous environment of the receptor.

The experimentally determined contacts between residues of the receptor N-ted and VIP peptide constitute a set of restrictive constraints severely limiting the possible locations of VIP in the binding site. The most interesting aspects of the docking procedure coupled to molecular dynamics concern the orientation of the bound VIP peptide with respect to the binding interface and the intermolecular interaction network implied in the binding process. As expected from site-directed mutagenesis studies (9), the interacting surface involves the C-terminal part of the N-ted of the receptor. The interface includes the last antiparallel β-sheet comprising residues Gln80–Leu84 (β3) and Val101–Cys105 (β4) and the unstructured C-terminal Trp110–Cys122 segment anchored to the β3 strand via the Cys86–Cys122 disulfide bond. The VIP peptide lies on the plane defined by the three aforementioned segments (Fig. 6). The surface contact area between VIP and the N-ted of the receptor is 800 Å². Since the surface area of VIP is 2755 Å², its surface of contact with the N-ted of the receptor represents 29% of the surface of the peptide ligand. The structure of VIP in the docking model presents a slight but distinctive kink in the middle of the helix. As a result, the first N-terminal part of the helix is parallel to the β sheet direction, whereas the C-terminal part is tilted at about 20°. According to our previous description of the free VIP conformation, the hydrophobic

A. Couvineau, unpublished observations.
patch (Leu\textsuperscript{13}–Met\textsuperscript{17}) contacts the β3 strand, forming a hydrophobic cluster with the bulky side chain of Val\textsuperscript{83} whereas the opposite patch (Val\textsuperscript{19}–Leu\textsuperscript{23}) points outward. This first intermolecular anchor is strengthened by an electrostatic interaction between the VIP-Lys\textsuperscript{21} side chain and the hVPAC1 receptor-Val\textsuperscript{83} backbone CO. The VIP C-terminal segment exhibits a second anchor revealed by a hydrogen bond between the Asn\textsuperscript{21} side chain of VIP and the backbone NH of Cys\textsuperscript{122} of the receptor, the latter being involved in the disulfide bond with Cys\textsuperscript{166}. These two anchors can be readily related to the VIP helix tilt mentioned above. Four VIP side chains to VPAC backbone CO groups complement the intramolecular interaction network: Arg\textsuperscript{14} (VIP)-Trp\textsuperscript{110} (receptor), Arg\textsuperscript{14} (VIP)-Thr\textsuperscript{111} (receptor), Lys\textsuperscript{21} (VIP)-Pro\textsuperscript{115} (receptor), and Ser\textsuperscript{25} (VIP)-Ile\textsuperscript{120} (receptor). This is consistent with the alanine scanning of VIP, in particular for Arg\textsuperscript{14} and Lys\textsuperscript{21}, the mutation of which into alanine results in important decreases of binding affinity (12). This is also in good agreement with mutagenesis data on the receptor since mutations of Trp\textsuperscript{110} and Pro\textsuperscript{115} into alanine decrease binding affinity of the hVPAC1 receptor for VIP (9).

The docking of VIP into the N-ted of the receptor shows that the residues Ph\textsuperscript{6}, Tyr\textsuperscript{22}, and Asn\textsuperscript{48} of VIP line up with the labeled residues determined by photoaffinity experiments in the receptor N-ted, i.e. Asp\textsuperscript{107}, Gly\textsuperscript{116}, and Cys\textsuperscript{122}, respectively. It further points out two prominent features: (i) the site of interaction of VIP with the receptor N-ted and the 3 asparagine residues bearing the large carboxy- ...

Acknowledgments—We thank Flavio Toma for the use of the 600-MHz Bruker NMR spectrometer in the Laboratoire de Structure et Reconnaissance des Biomolécules, Université d’Evry Val d’Essonne, France. We thank Maqing Dong (Mayo Clinic in Scottsdale, Scottsdale, AZ) for technical advice in sequencing experiment. We thank Marylin Perrin (The Salk Institute for Biological Studies, La Jolla, CA) for helpful discussion.

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