Diffuse Pharmacophoric Domains of Vasoactive Intestinal Peptide (VIP) and Further Insights into the Interaction of VIP with the N-terminal Ectodomain of Human VPAC1 Receptor by Photoaffinity Labeling with [Bpa\(^6\)]-VIP*  

Yossan-Var Tanž, Alain Couvineau, and Marc Laburthe§

From the INSERM U410, Neuroendocrinologie et Biologie Cellulaire Digestives, Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine Xavier Bichat, Paris F-75018, France

The widespread 28-amino acid neuropeptide vasoactive intestinal peptide (VIP) exerts its many biological effects through interaction with serpentine class II G protein-coupled receptors named VPAC receptors. We previously provided evidence for a physical contact between the side chain at position 22 of VIP and the N-terminal ectodomain of the hVPAC1 receptor (Tan, Y. V., Couvineau, A., Van Rampelbergh, J., and Laburthe, M. (2003) J. Biol. Chem. 278, 36531–36536). We explored here the contact site between hVPAC1 receptor and the side chain at position 6 of VIP by photoaffinity labeling. The photoreactive para-benzoyl-l-Phe (Bpa) was substituted for Phe\(^6\) in VIP resulting in [Bpa\(^6\)]-VIP, which was shown to be a hVPAC1 receptor agonist in Chinese hamster ovary cells stably expressing the recombinant receptor. After obtaining the covalent \(125\)I-[Bpa\(^6\)]-VIP-hVPAC1 receptor complex, it was sequentially cleaved by cyanogen bromide, peptide \(N\)-glycosidase F, endopeptidase Glu-C, and trypsin, and the cleavage products were analyzed by electrophoresis. The data demonstrated that \(125\)I-[Bpa\(^6\)]-VIP were covalently attached to the short 104–108 fragment within the N-terminal ectodomain of the receptor. The data were confirmed by creation of a receptor mutant with new CNBr cleavage site. In a three-dimensional model of the receptor N-terminal ectodomain, this fragment was located on one edge of the putative VIP-binding groove and was adjacent to the fragment covalently attached to the side chain at position 22 of VIP. Altogether these data showed that the central part of VIP, at least between Phe\(^6\) and Tyr\(^{22}\), interacts with the N-terminal ectodomain of the hVPAC1 receptor.

The neuropeptide vasoactive intestinal peptide (VIP)\(^1\) is present in both central and peripheral nervous systems as well as in immune cells (1). It controls a large array of biological functions in the brain and peripheral organs (1) and was shown recently to exert potent anti-inflammatory actions (2). The two cloned VIP receptors also bind with high affinity another neuropeptide, the pituitary adenyl cyclase-activating peptide, and have been named VPAC\(^2\) receptors thereby (3). They are class II G protein-coupled receptor-like receptors for all peptides structurally related to VIP and also receptors for parathyroid hormone, calcitonin, and corticotropin-releasing factor (3).

The VPAC1 receptor is prototypic of class II G protein-coupled receptors and has been extensively studied by molecular biology techniques including site-directed mutagenesis and molecular chimerism (for review see Ref. 3). These studies made it possible to delineate the receptor domains involved in high affinity VIP binding (3), selectivity toward some natural peptide agonists (4, 5) and also activation of adenyl cyclase (6). With respect to VIP binding, it appeared that the N-terminal ectodomain of the receptor plays a crucial role, although it is not sufficient to ensure high affinity (3). A three-dimensional model of the N-terminal ectodomain of the hVPAC1 receptor has been developed suggesting the existence of a VIP-binding groove within this domain (7). Despite these extensive studies of the structure-function relationship of hVPAC1 receptor, the physical sites of interaction between VIP and its receptors had remained elusive until recent photoaffinity showing labeling experiments that the side chain of position 22 of VIP is in direct contact with one edge of the putative binding groove in the N-terminal ectodomain (8).

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 (9). In this context, we further explored the contact sites between VIP and the hVPAC1 receptor by incorporating a photoactivable benzophenone group on the side chain at position 6 of VIP. This was done by substituting para-benzoyl-l-Phe (Bpa) for phenylalanine 6. This site of incorporation was selected for several reasons: (i) Phe\(^6\) is important for the biological activity of VIP (9) and is strictly conserved in all natural hormones structurally related to VIP (1). (ii) The substitution of Bpa for phenylalanine keeps an aromatic residue, and we expected that, even though Phe\(^6\) is important for biological activity, the [Bpa\(^6\)]-VIP probe should keep reasonable affinity for the receptor. (iii) Position 6 and the previously explored position 22 (8) are at the two ends of the central a-helical domain of VIP (9, 10). We report here that the amino acid in...
position 6 of VIP is in the environment of the short sequence 104–108 within the N-terminal ectodomain of the receptor when the photoaffinity probe is bound to the receptor.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction enzymes and culture medium were obtained from Invitrogen. Eucaryotic expression vector was from Clontech (Palo Alto, CA). DNA Sequenase kit and radioactive reagents were from Amersham Biosciences. The site-directed mutagenesis kit was from Promega (Charbonnières, France). Synthetic oligonucleotides were from Invitrogen. The human VPAC1 receptor cDNA was cloned in our laboratory (11). A receptor construct containing an inserted FLAG sequence between the N- and C-termini of the native receptor and the green fluorescent protein (12) was used in all studies. It has the same phenotype as the wild-type receptor with respect to VIP binding and activation of adenyl cyclase (12). This construct or derived mutants were stably transfected in CHO cells as described (9). The CHO-F7 clone expressing the human VPAC1 receptor construct (β2-m, 5 pmol protein; Kd, 0.6 nM VIP) was used in most experiments. VIP and the photolabile VIP probe, [Ala17, para-benzoyl-L-Phe]–VIP–88 referred to as [Bpa6]–VIP were obtained by custom synthesis from Neo-System (Strasbourg, France). 125I–VIP, 125I–[Bpa6]–VIP, and 125I–[Bpa6]–VIP were prepared and purified in our laboratory as previously described (9). All of the highly purified chemicals used were from Sigma.

**Cell Culture and Membrane Preparation**—The CHO-F7 cells expressing the wild-type VPAC1 receptor construct and CHO cells expressing stably transfected receptor mutant were utilized as sources of receptor for this study and were cultured in Ham’s F-12 medium supplemented with 10% decomplemented fetal calf serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin in a humidified atmosphere containing 95% air and 5% CO2 at 37°C. The cells were grown to confluency. After removing the culture medium, the attached cells were washed twice with phosphate-buffered saline and then harvested with a rubber policeman and centrifuged for 10 min at 3,000 × g. The cell pellet was exposed for 30 min at 4°C on ice at a distance of ~2 cm. After 40 min of UV exposure, the cells were collected into individual 1.5-ml Eppendorf tubes, washed twice with 10 ml HEPES containing 25 mM glycine, 75 mM NaCl buffer, pH 2.5, and washed one time with 20 ml HEPES. The photolabeled receptors were then analyzed directly by electrophoresis or after chemical or enzymatic cleavage.

**Chemical Cleavage of Photoaffinity-labeled VPAC1 Receptor Constructs**—The photolabeled receptors in cells were incubated overnight in darkness with 10 mg/ml CNBr in 80% formic acid at room temperature. The CNBr present in the reaction mixture was then removed by Waters C18 Sep-Pak purification. After extensive washing of the Sep-Pak cartridge with 0.1% acetonitrile, CNBr–derivatized and unlabelled fractions were eluted with 2 ml of 100% acetonitrile. The fractions were counted for radioactivity and evaporated under vacuum (Speed-Vac). The resulting material was either analyzed directly by electrophoresis or after enzymatic treatments (see below). After migration, labeled bands were visualized by autoradiography. For further enzymatic treatments the bands were cut out, electroeluted (model 422 electroeluter, Bio-Rad), and lyophilized.

**Enzymatic Treatments**—For protein deglycosylation, the material obtained after CNBr treatment was incubated for 2 h at 37°C with PNGase F (3 units/ml) as described (14). Endopeptidase Glu-C digestion of proteins in this material was obtained by incubation for 6 h at 25°C in 25 mM ammonium carbonate, 0.01% SDS, 5% acetonitrile, pH 7.8, as described (15). The cleavage of proteins was performed for 2 h at 37°C in 0.1 M MOPS buffer, pH 7.0.

**Electrophoresis and Autoradiography**—Products of cleavage were resolved on NuPAGE 4–12% Bis-Tris Gel using MES SDS running buffer system from Invitrogen performed according to the method of Lammli (16) under reducing conditions in the presence of 20 mM dithiothreitol. After electrophoresis, the gels were dried and exposed to x-ray film for 3–10 days with intensifying screens at −80°C as described (14). The apparent molecular masses of radiolabeled receptor fragments were determined by interpolation on a plot of the mobility of the protein molecular weight markers colored with Rainbow™ from Amersham Biosciences or protein ladder pretrained with Benchmark™ from Invitrogen versus the log values of their masses.

**RESULTS AND DISCUSSION**

In this study, the synthetic [Bpa6]–VIP photoaffinity probe that incorporated the photolabile residue para-benzoyl-L-Phe to replace Phe6 of VIP was first evaluated for its ability to interact specifically with the human VPAC1 receptor stably expressed in CHO-F7 cells. [Bpa6]–VIP has an efficacy similar to that of native VIP in stimulating adenyl cyclase activity but a lower potency with EC50 values of 1.10 and 0.05 nM, respectively (Fig. 1). In the binding assay, [Bpa6]–VIP was also less potent than native VIP in inhibiting 125I–VIP binding with Ki values of 36 and 0.6 nM, respectively. These data indicate that the [Bpa6]–VIP probe is a low potency VPAC1 receptor agonist which is instrumental for photoaffinity labeling of the receptor because its affinity is not too much decreased. The fact that the addition of a para-benzoyl group on Phe6 of VIP results in a decrease of affinity for VIP receptor is in agreement with the important role of Phe6 in VIP activity as previously demonstrated by alanine scanning of the neuropeptide (9). As stated under “Materials,” the photoaffinity probe is actually Ala17 in addition to Bpa6. The replacement of Met17 by Ala was employed to simplify subsequent analysis following CNBr cleavage (see below) and was previously shown to keep intact the binding affinity for the hVPAC1 receptor (9).

After incubation of CHO-F7 cells with the radiiodinated 125I–[Bpa6]–VIP probe, labeled cells were exposed to UV to create a covalent bond between the probe and the receptor. SDS-PAGE analysis of proteins revealed the existence of a single labeled band that completely disappeared when an excess of cold VIP was co-incubated with the radiiodinated probe (Fig. 2). This band migrated at a molecular mass of 95 kDa (Fig. 2) in good agreement with the expected mass (92 kDa) considering the previously characterized (14) mass of the glycosylated VPAC1 receptor (64 kDa), the presence of the green fluorescent...
experiments. For the sake of clarity the error bars
an excess of cold VIP (1
hVPAC1 receptor by incubation of the labeled probe in the presence of
) to treatment with PNGase F. Inhibition of photoaffinity labeling of
1
VPAC1 receptor domain to which 125I-[Bpa6-VIP] is covalently
iciently and specifically label the human VPAC1 receptor.
lane 3
lane
preparation derived from these cells was subjected (lane 2
/H9262
VIP-stimulated (1
moiety on each of the
kDa) and the probable presence of the 9-kDa carbohydrate
ment with PNGase F (Fig. 3), indicating that it is heavily
tor with the 125I-[Bpa6-VIP] probe.
Fig. 1. Binding and adenylyl cyclase activity assays of the [Bpa6]-VIP probe. Left panel, competitive inhibition of 125I-VIP binding to
CHO-F7 cell membranes expressing wild-type hVPAC1 receptor by VIP and [Bpa6]-VIP. The data are expressed as percentages of initial specific
binding in the absence of competitor. Right panel, effect of increasing concentrations of VIP and [Bpa6]-VIP on adenylyl cyclase activity in CHO-F7
cell membranes. The data are expressed as percentages of maximal stimulation above basal obtained with 1 µM native VIP. The basal and
VIP-stimulated (1 µM) enzyme activities are 12 ± 1 and 136 ± 13 pmol cAMP/min/mg of proteins. All data are the means ± S.E. of three
experiments. For the sake of clarity the error bars are not indicated. They are always less than 10% of the mean values. ○, VIP; ●, [Bpa6]-VIP.
protein (25 kDa) at C terminus of the receptor, and the co-
valently attached [Bpa6]-VIP (3 kDa) in the complex. The 95-
kDa labeled band shifted to a 70-kDa band after deglycosyla-
tion with PNGase F (Fig. 1). After PNGase F treatment, the labeled probe in the presence of
an excess of cold VIP (1 µM) is shown in lane 2. The labeled proteins
were resolved on NuPAGE 4–12% Bis-Tris Gel followed by autoradiog-
raphy. See “Experimental Procedures” for details. MW, molecular mass.

Fig. 2. Photoaffinity labeling of the wild-type hVPAC1 recep-
tor with the 125I-[Bpa6-VIP] probe. Receptors expressed in CHO-F7
cells were photoaffinity-labeled with 125I-[Bpa6-VIP] and a membrane
preparation derived from these cells was subjected (lane 3) or not (lane 1) to treatment with PNGase F. Inhibition of photoaffinity labeling of
hVPAC1 receptor by incubation of the labeled probe in the presence of
VIP is represented by the Trp
–
Met
127 (Table I), whose theoretical mass is 2.6 kDa. Indeed,
73 – 93 sequence or 4.3 kDa for the 94–137 sequence. It is consis-
tent the removal of the 9-kDa carbohydrate moiety known to be linked to Asn69 and Asn100. The mutant F93M was constructed and stably ex-
pressed in CHO cells. It bound VIP with an affinity similar to that of the wild-type receptor (Fig. 4). The Kd for VIP was 1.5
and 0.6 nM for the F93M mutant and wild-type receptor, re-
spectively. After incubation of the F93M mutant with 125I-
-[Bpa6-VIP] and CNBr treatment of proteins, a 18-kDa labeled
band was observed instead of a 30-kDa band for the wild-type
receptor (Fig. 5). The 12-kDa mass difference observed between
the wild-type receptor and mutant cannot be simply accounted
for by the shortening of the peptide fragment e.g. 2.6 kDa for the
67–93 sequence or 4.3 kDa for the 94–137 sequence. It is consis-
tent the removal of the 9-kDa carbohydrate moiety

We proceeded with our study with the identification of the
VPAC1 receptor domain to which 125I-[Bpa6-VIP] is covalently
attached. For that purpose the 95-kDa band corresponding to
125I-[Bpa6-VIP]:hVPAC1R complex was subjected to a series of
c hemical and enzymatic cleavages. Cyanogen bromide cleavage of the 95-kDa band generated a single 30-kDa labeled band
(Fig. 3). This band was further shifted to 11 kDa after treat-
ment with PNGase F (Fig. 3), indicating that it is heavily
glycosylated, most probably at two consensus N-glycosylation
sites. Considering the molecular mass of the attached probe (3
kDa) and the probable presence of the 9-kDa carbohydrate
moiety on each of the N-glycosylation sites (14), the best can-
didate corresponding to the covalent attachment of 125I-[Bpa6-
VIP] is represented by the Trp
–
Met
137 receptor fragment (Table I) present in the N-terminal extracellular
domain of the VPAC1 receptor. This fragment contains two N-
glycosylation sites on Asn69 and Asn100 (Table I). To further
validate the Trp
–
Met
137 receptor fragment as the region of interaction with the 125I-[Bpa6-VIP] probe, we created a new
CNBr cleavage site by substituting a methionine for Phe93 by
site-directed mutagenesis. This site was chosen because it
stands between the two N-glycosylation sites on Asn69
and Asn100. The mutant F93M was constructed and stably ex-
pressed in CHO cells. It bound VIP with an affinity similar to
that of the wild-type receptor (Fig. 4). The Kd for VIP was 1.5
and 0.6 nM for the F93M mutant and wild-type receptor,
respectively. After incubation of the F93M mutant with 125I-
-[Bpa6-VIP] and CNBr treatment of proteins, a 18-kDa labeled
band was observed instead of a 30-kDa band for the wild-type
receptor (Fig. 5). The 12-kDa mass difference observed between
the wild-type receptor and mutant cannot be simply accounted
for by the shortening of the peptide fragment e.g. 2.6 kDa for the
67–93 sequence or 4.3 kDa for the 94–137 sequence. It is consis-
tent the removal of the 9-kDa carbohydrate moiety

Moreover, as shown in Fig. 5, the deglycosylation of the 18-kDa
labeled band with PNGase F generated a fragment migrating
at an approximate molecular mass of 9 kDa, corresponding to
the removal of a single carbohydrate moiety. Although these
experiments clearly confirmed that the 67–137 receptor frag-
ment was the site of covalent binding of the 125I-[Bpa6-VIP]
probe, alone they did not allow to determine which one of the
67–93 or 94–137 fragment was labeled in the F93M receptor
mutant. To discriminate between the two fragments, the 18-
kDa labeled band (see above) was electroeluted and incubated
with trypsin. After trypsin treatment, the labeled material
migrated as a narrow 4-kDa band (Fig. 5). Considering that
the 125I-[Bpa6-VIP] probe itself is cleaved by trypsin (Table II),
generating the 1.4 kDa 1–12 VIP fragment bearing both the
para-benzoyl-L-Phe6 and the 125I-Tyr10, the only receptor frag-
ment compatible with the labeling of a 4-kDa band is fragment
104–127 (Table I), whose theoretical mass is 2.6 kDa. Indeed,
the mass of this fragment (2.6 kDa) plus the mass of the
cleaved probe (1.4 kDa) corresponds to the 4-kDa estimated for
the labeled band after trypsin cleavage (Fig. 5). All of the other
receptor fragments generated by trypsin cleavage (Table I) are
much too small to be compatible with the experimental data.

To further delineate the site of covalent binding of the affin-
ity probe, the 30-kDa CNBr-generated fragments (Fig. 3) obtained after photoaffinity labeling of the wild-type receptor was digested with endopeptidase Glu-C (Fig. 3), which under our experimental conditions cleaves proteins at the C-terminal side of Glu residues with the notable exception of the Glu-Pro sequence (15). Endopeptidase Glu-C treatment of the 30-kDa band generated a 25-kDa band representing the receptor segment 67–108 that contains two N-glycosylation sites on Asn 69 and Asn 100 (Table I). Accordingly, this band was further shifted to 8 kDa upon treatment with PNGase F (Fig. 3). These data were thus consistent with the covalent binding of the 125I-[Bpa 6-VIP] probe to the Trp 67-Glu 108 glycosylated receptor fragment (Table I). Then trypsin, which cleaves at the C-terminal side of Lys and Arg residues, was used to further narrow the domain of covalent binding of the [Bpa 6]-VIP probe. As shown in Fig. 3, trypsin digestion of the 30-kDa band resulted in a nonglycosylated fragment migrating at 4 kDa. The mass of trypsin-generated product strongly supported the possibility that the Ser 104–Lys 127 fragment is actually the site of attachment of [Bpa 6]-VIP (Table I) as demonstrated above with the F93M receptor mutant (Fig. 5). Finally, the CNBr-generated 30-kDa band was sequentially digested with PNGase F, endopeptidase Glu-C, and trypsin. This sequential treatment generated a 2-kDa labeled band. This fragment was compatible with the covalent attachment of the probe to the Ser 104–Glu 108 sequence of the hVPAC1 receptor. Indeed, its mass of 2 kDa was very similar to the mass of the receptor fragment (0.55 kDa) plus the mass of the trypsin-cleaved probe (1.4 kDa; see above and Table II).

Our data show that there is a spatial approximation between Phe 6 of VIP and the 104–108 sequence within the N-terminal ectodomain of the human VPAC1 receptor. In a previous complete alanine scanning of VIP (9), the residues comprising VIP have been characterized into one of three different categories. Phe 6 was placed in category 2, which is defined as residues whose substitution into alanine is associated with a significant decrease of the binding affinity and biological potency of the corresponding analog as well as a change of the predicted
structure as compared with native VIP (9). Consequently, we speculated that the decreased affinity of the F6A VIP mutant was due to its altered structure, although the possible direct involvement of the category 2 residues, including Phe\textsuperscript{6}, in VIP binding to receptor cannot be ruled out. In this context, we tried to identify the individual residue, or residues, in the short 104–108 receptor fragment that may interact with residue 6 of VIP by individually mutating to alanine between Glu\textsuperscript{36} and Pro\textsuperscript{115} in the N-terminal ectodomain. As shown in Table III, all of the mutants but C105A bound VIP with the same affinity as the wild-type receptor. In sharp contrast, no binding could be detected with C105A bound VIP with the same affinity as the wild-type receptor as shown in lane J. The labeled proteins were resolved on NuPAGE 4–12% Bis-Tris Gel followed by autoradiography. See “Experimental Procedures” for details. MW, molecular mass.

Table II
Theoretical fragments generated by trypsin cleavage of \([\text{Bpa}^\text{a}]-\text{VIP}\) probe

| Mass (kDa) | Position | Peptide sequence |
|----------|----------|-----------------|
| 1425.6593 | 1–12 | HSDAV pBz-F TDNYTR\textsuperscript{b} |
| 836.4512 | 22–28 | YLNSILN |
| 516.3140 | 16–20 | QAAVK\textsuperscript{c} |
| 288.2030 | 13–14 | LR |

\textsuperscript{a} Theoretical mass of receptor fragments calculated from the amino acid sequence.

\textsuperscript{b} pBz-F, Phe\textsuperscript{6} of VIP has been replaced by para-benzoyl-L-Phe.

\textsuperscript{c} Ala has been substituted for Met\textsuperscript{6} of VIP to abolish a CNBr cleavage site in the peptide.

hVPAC1 receptor as the contact region between receptor and the side chain at position 6 of VIP. This is in good agreement with previous mutagenesis studies suggesting that the N-terminal ectodomain is a major site of VIP binding (reviewed in Ref. 3). Indeed, mutagenesis showed that the individual mutation of 12 amino acids into alanine between Glu\textsuperscript{36} and Pro\textsuperscript{115} resulted in important or total loss of VIP binding (3, 7, 18–20). It is quite interesting to note that this large segment revealed by mutagenesis studies encompasses the short Ser\textsuperscript{104}–Glu\textsuperscript{108} sequence of the receptor shown here by photoaffinity labeling to be the contact region of the side chain at position 6 of VIP. We previously developed a structural model of the N-terminal ectodomain of the VPAC1 receptor by sequence homology with a yeast lipase (7). This model showed that residues that are important for VIP binding, as demonstrated by mutagenesis, are gathered around an electronegative groove with an outspanning shell of three tryptophan residues at one end (7). In this context, the fact that the present study demonstrated that 125I-[\text{Bpa}^\text{a}]-\text{VIP}\) covalently attached to the Ser\textsuperscript{104}–Glu\textsuperscript{108} sequence of the receptor shown here by photoaffinity labeling to be the contact region of the side chain at position 6 of VIP is shown in black within yellow balls.

Fig. 6. Ribbon representation of the three-dimensional model of the hVPAC1 (1–139) receptor N-terminal ectodomain highlighting our current knowledge of its interaction with VIP. This model (3, 7) supports the presence of a binding groove. The receptor fragment (residues 104–108) covalently labeled by 125I-[\text{Bpa}^\text{a}]-\text{VIP}\) is shown in red. The receptor fragment (residues 109–120) covalently labeled by 125I-[\text{Bpa}^\text{a}]-\text{VIP}\) (8) is shown in yellow. VIP is shown in orange, and positions 6 and 22 in VIP are numbered in black within yellow balls.

Table III
Binding parameters of hVPAC1 receptor mutants

| Receptor constructs | $K_d$ \textsuperscript{a} (nM) | $B_{\text{max}}$ (pmol/mg of proteins) |
|---------------------|-----------------------------|-------------------------------|
| Wild type           | 0.41 ± 0.03                 | 4.41 ± 0.79                   |
| S104A               | 0.34 ± 0.04                 | 6.38 ± 0.02                   |
| C105A               | ND                          | ND                            |
| T106A               | 0.42 ± 0.05                 | 3.37 ± 0.92                   |
| D107A               | 0.45 ± 0.09                 | 4.78 ± 0.80                   |
| E108A               | 0.32 ± 0.05                 | 1.60 ± 0.04                   |

\textsuperscript{a} $K_d$ and $B_{\text{max}}$ were determined as described under “Experimental Procedures”.

\textsuperscript{b} ND, not detected.
This is illustrated in Fig. 6, which highlights the position of the two contact segments and also tentatively positions VIP in the binding groove taking into account the previous modelization (9) and NMR (23) studies of the peptide, which supported the idea that VIP has a central α-helix with a kink and less structured C- and N-terminal ends. Our data (present paper and Ref. 8) are consistent with a model in which the central structured C- and N-terminal ends. Our data (present paper and also to identify new anchor points of the N-terminal probes of VIP are needed to further delineate the sites of binding groove within the N-terminal ectodomain of the hVPAC1 receptor. Additional studies with new photoaffinity probes of VIP are needed to further delineate the sites of hVPAC1 receptor N-terminal ectodomain and the side chain at position 6 of VIP. Together with a previous photoaffinity labeling study –

ACKNOWLEDGMENT—We thank Jean-Jacques Lacapère (INSERM U410) for helpful discussion regarding molecular modelization.

REFERENCES

1. Sherwood, N. M., Krueckl, S. L., and McRory, J. E. (2000) Endocr. Rev. 21, 619–670
2. Delgado, M., Abad, C., Martinez, C., Juarranz, M. G., Arranz, A., Gomariz, R. P., and Leceta, J. (2002) J. Mol. Med. 80, 16–24
3. Laburthe, M., Couvineau, A., and Marie, J. C. (2002) Receptors Channels 8, 137–153
4. Couvineau, A., Rouyer-Fessard, C., Maoret, J. J., Gaudin, P., Nicole, P., and Laburthe, M. (1996) J. Biol. Chem. 271, 12785–12800
5. Du, K., Couvineau, A., Rouyer-Fessard, C., Nicole, P., and Laburthe, M. (2002) J. Biol. Chem. 277, 37016–37022
6. Couvineau, A., Lacapère, J. J., Tan, Y. V., Rouyer-Fessard, C., Nicole, P., and Laburthe, M. (2003) J. Biol. Chem. 278, 24759–24766
7. Lins, L., Couvineau, A., Rouyer-Fessard, C., Nicole, P., Maoret, J. J., Benhamed, M., Brasseur, R., Thomas, A., and Laburthe, M. (2001) J. Biol. Chem. 276, 10153–10160
8. Tan, Y. V., Couvineau, A., Van Rampelbergh, J., and Laburthe, M. (2003) J. Biol. Chem. 278, 36551–36556
9. Nicole, P., Lins, L., Rouyer-Fessard, C., Drouet, C., Fucrland, P., Thomas, A., Couvineau, A., Martinez, J., Brasseur, R., and Laburthe, M. (2000) J. Biol. Chem. 275, 24003–24012
10. Fry, D. C., Madison, V. S., Bohn, D. R., Greeley, D. N., Toome, V., and Wegrzynski, B. B. (1989) Biochemistry 28, 2399–2409
11. Couvineau, A., Rouyer-Fessard, C., Darnoul, D., Maoret, J. J., Carrero, I., Ogier-Denis, E., and Laburthe, M. (1994) Biochem. Biophys. Res. Commun. 200, 769–776
12. Gaudin, P., Maoret, J. J., Couvineau, A., Rouyer-Fessard, C., and Laburthe, M. (1998) J. Biol. Chem. 273, 4990–4996
13. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
14. Couvineau, A., Fabre, C., Gaudin, P., Maoret, J. J., and Laburthe, M. (1996) Biochemistry 35, 1745–1752
15. Sorensen, S. B., Sorensen, T. L., and Breddam, K. (1991) FEBS Lett. 294, 195–197
16. Laemmli, U. K. (1970) Nature 227, 680–685
17. Gaudin, P., Couvineau, A., Maoret, J. J., Rouyer-Fessard, C., and Laburthe, M. (1995) Biochem. Biophys. Res. Commun. 211, 901–908
18. Couvineau, A., Gaudin, P., Maoret, J., Rouyer-Fessard, C., Nicole, P., and Laburthe, M. (1995) Biochem. Biophys. Res. Commun. 206, 246–252
19. Nicole, P., Du, K., Couvineau, A., and Laburthe, M. (1998) J. Pharmacol. Exp. Ther. 284, 744–750
20. Nicole, P., Maoret, J., Couvineau, A., Momaney, F. A., and Laburthe, M. (2000) Biochem. Biophys. Res. Commun. 276, 654–659
21. Solano, R. M., Langer, J., Perret, J., Vertongen, P., Juarranz, M. G., Robberec, P., and Waelbroeck, M. (2001) J. Biol. Chem. 276, 1084–1088
22. Dong, M., and Miller, L. J. (2002) Receptors Channels 8, 189–200
23. Cherev, M. (2002) Receptors Channels 8, 219–224
24. Gensure, R. C., Gardella, T. J., and Juppner, H. (2001) J. Biol. Chem. 276, 28650–28658
25. Gaylinn, B. D. (2002) Receptors Channels 8, 155–162
Diffuse Pharmacophoric Domains of Vasoactive Intestinal Peptide (VIP) and Further Insights into the Interaction of VIP with the N-terminal Ectodomain of Human VPAC1 Receptor by Photoaffinity Labeling with [Bpa6]-VIP

Yossan-Var Tan, Alain Couvineau and Marc Laburthe

J. Biol. Chem. 2004, 279:38889-38894.
doi: 10.1074/jbc.M404460200 originally published online July 6, 2004