Elucidation of Vasoactive Intestinal Peptide Pharmacophore for VPAC₁ Receptors in Human, Rat, and Guinea Pig

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ABSTRACT

Vasoactive intestinal peptide (VIP) is a neurotransmitter involved in a number of pathological and physiological processes. VIP is rapidly degraded and simplified stable analogs are needed. VIP’s action was extensively studied in rat and guinea pig. However, it is largely unknown whether its pharmacophore in these species resembles human. To address this issue we investigated the VIP pharmacophore for VPAC₁, the predominant receptor subtype in cancers and widely distributed in normal tissues by using alanine and D-amino acid scanning. Interaction with rat, guinea pig, and human VPAC₁ was assessed using transfected Chinese hamster ovary (CHO) and PANC1 cells and cells possessing native VPAC₁. Important species differences existed in the VIP pharmacophore. The human VPAC₁ expressed in CHO cells, which were used almost exclusively in previous studies, differed markedly from the native VPAC₁ in T47D cells. The most important amino acids for determining affinity are His¹, Asp³, Phe⁶, Arg¹², Arg¹⁴, and Leu¹⁹, Ser², Asp⁸, Asn⁹, Thr¹¹, Val¹⁹, Asn²⁴, Ser²⁵, Leu²⁷, and Asn²⁸ are not essential for high-affinity interaction/activation. [Ala²,8,9,11,19,24,25,27,28]VIP, which contained 11 alanines, was synthesized and it was equipotent to VIP at VPAC₁ receptors in all species and was metabolically stable. Our results show in any design of simplified VIP analogs for VPAC₁, it will be important to consider species differences and it is essential to use transfected systems that reflect the native receptor’s pharmacophore. Last, with our results a simplified, metabolically stable VIP analog was identified that should be useful as a prototype for design of selective agonists/antagonists that could be useful therapeutically.

Vasoactive intestinal peptide (VIP) is a 28-amino acid peptide widely distributed in both the central nervous system and peripheral tissues where it functions as a neurotransmitter and neuromodulator (Dockray, 1994). VIP is involved as a neural regulator of pancreatic and intestinal secretion, gastrointestinal motility, and blood flow, and also functions in a similar capacity in the cardiovascular, respiratory, and urogenital tracts (Dockray, 1994). VIP also has important growth effects (Muller et al., 1995), effecting the growth of cancers (prostate, lung, pancreatic, breast) (Moody, 1996; Jiang et al., 1997; Moody et al., 1998) as well as normal tissues (Gozes and Brenneman, 2000). Recent reports propose a role of VIP in a number of clinically important areas, including asthma (Bolin et al., 1995); treatment of impotence (Edvinsson, 2000); having a neuroprotective effect that could be used to treat Alzheimer’s disease (Gozes and Brenneman, 2000) or Parkinson’s disease (Offen et al., 2000); and having potent anti-inflammatory effects that could be useful in the treatment of septic shock, Crohn’s disease, and rheumatoid arthritis (Gomariz et al., 2001; Said, 1996). In addition VIP receptors are highly expressed in a number of cancers, including those of the breast and gastrointestinal tract, and it has been shown these can be used to localize these tumors by imaging methods and, therefore, their presence may be important in various antitumor treatments (Virgolini, 1997).

VIP is structurally similar to pituitary adenylate cyclase-activating peptides, secretin, peptide histidine isoleucine, glucagon, glucagon-like peptides, gastric inhibitory polypeptide, and growth hormone-releasing factor (Dockray, 1994). Two receptors that share 50% homology, VPAC₁ and VPAC₂, mediate the actions of VIP (Ulrich et al., 1998). Both receptors...
tors are members of the G protein-coupled, heptahelical superfam-
ily (Ulrich et al., 1998) but these differ in their distribution (Waschek et al., 1995; Reubi et al., 2000) and pharmacology (Harmar et al., 1998).

The true role of VIP in the above-mentioned processes is poorly understood for a number of reasons. These include a lack of potent antagonists, lack of simplified analogs that are resistant to degradation and could be used in vivo, and a lack of understanding of the VIP pharmacophore for its receptor to use to design simplified analogs. Although there are numerous studies of the pharmacology of VIP and related peptides (O’Donnell et al., 1989; Jensen, 1994; Bolin et al., 1995; Nicole et al., 2000), these studies provide limited information on the VIP pharmacophore. The principal reason is that except for one study (Nicole et al., 2000), all of these studies were performed before the recognition that two subtypes of VIP receptors mediate VIP’s actions and are frequently present in the same tissue (Usdin et al., 1994; Waschek et al., 1995; Ulrich et al., 1998; Ito et al., 2000). Furthermore, many studies were performed using receptor preparations from guinea pigs and rodents. In some studies (Robberecht et al., 1988; O’Donnell et al., 1991; Bolin et al., 1995), but not others (Leroux et al., 1994; Ito et al., 2000), the VIP pharmacophore in these species is reported to be similar to human receptors. Even in studies on VIP receptor pharmacology performed since the VIP receptor structures have been identified, by using transfected VIP receptors, it has not been established the pharmacology revealed by the transfected cell systems used faithfully reflects that seen with the native VIP receptor. Studies demonstrate that the entire molecule is required for high-affinity interaction with VIP receptors (Jensen, 1994). Furthermore, in vivo VIP has a half-life of less than 1 min (Domschke et al., 1978). Developing simplified, stable VIP analogs with high affinity for VIP receptors would be useful as prototypes to develop more stable analogs, selective receptor analogs that interact with one of the two G protein-coupled receptors mediating VIP’s actions (Harmar et al., 1998), or for in vivo physiological and pathological studies. However, to eventually develop such analogs it is essential to understand VIP’s pharmacophore in humans and different species commonly used in experimental studies.

Therefore, the goal of the present study was to determine the VIP pharmacophore for the VPAC1 receptor, which is the predominant subtype in cancers and widely distributed in normal tissues (Reubi et al., 2000) by assessing its interaction with the human VPAC1 and the VPAC1 from different species commonly used in laboratory studies. To accomplish this it was necessary to develop cell systems expressing the VPAC1-R whose pharmacology reflected that of the native receptor. We identified human, rat, and guinea pig cells containing only or >90% native VPAC1-R and prepared different cell lines that stably expressed VPAC1-Rs and determined the VPAC1-R pharmacophore in these VPAC1-R-containing cells by performing alanine and D-amino acid scans of VIP. Our results demonstrated that there were significant species differences in the VIP pharmacophore for the VPAC1 receptor and that the type of cell used for the stable expression of the VPAC1-R has a significant effect on the VIP pharmacophore. Furthermore, our results identify at least nine amino acids in VIP that we could replace with alanines to develop a simplified analog that was metabolically stable and retained high affinity for the VPAC1 in human, rat, and guinea pig.

Experimental Procedures

Materials. Male guinea pigs (100–150 g) were obtained from the Charles River Laboratories, Inc. (Wilmington, DE). Male Sprague-Dawley rats (80–100 g) were obtained from Taconic Farms (Germantown, NY). CHO cells, PANC1 human pancreatic cancer cells, H508 human colon cancer cells, T98G human glioblastoma cells, and Sup T1 human lymphoblastoma cells were obtained from American Type Culture Collection (Rockville, MD). T47D human breast cancer cells were a gift from Dr. Terry W. Moody (Cell and Cancer Biology Department, Medicine Branch, National Cancer Institute, Rockville, MD). Porcine VIP was purchased from Bachem Biosciences (King of Prussia, PA); purified collagenase (type CLSPA) from Worthington Biochemicals (Freehold, NJ); basal Eagle’s medium amino acid mixture, basal Eagle’s medium vitamin solution, fetal bovine serum, and LipofectAMINE transfection reagent from Invitrogen (Carlsbad, CA); geneticin (G418 sulfate) from Mediatech (Herndon, VA); pcDNA 3.1(+) and pcDNA 3.1(−) from Invitrogen; bacitracin, soybean trypsin inhibitor, and 1,3-dimethylxanthine (theophylline) from Sigma Chemical (St. Louis, MO); bovine serum albumin (BSA) fraction V from ICN Biomedicals (Aurora, OH); [125I]-VIP (2200 Ci/mmol) from PerkinElmer Life Sciences (Boston, MA); Na1,3,4,6-tetra-
chloro 3-octa-4,6-diphenylglycouril (IODO-GEN) from Pierce Chemical (Rockford, IL); and Phadebas amylase test reagent from Pharmacia Diagnostics (Piscataway, NJ). The standard incubation solution contained 24.5 mM HEPES, pH 7.45, 98 mM NaCl, 6 mM KCl, 2 mM KH2PO4, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 0.5 mM CaCl2, 1 mM MgCl2, 1% (w/v) BSA, 0.2% (w/v) soybean trypsin inhibitor, 1% (v/v) amino acid mixture, and 1% (v/v) essential vitamin mixture.

Preparation of Peptides. Native VIP has an alanine in position 4 and 18, so the remaining 26 amino acids were replaced sequentially. Therefore, 26 VIP analogs with a single alanine substitution in VIP numbered from the amino terminal amino acid position 1 to position 28 in the carboxyl terminus of VIP were synthesized. In addition, 28 VIP analogs with a single D-amino acid substitution in VIP numbered from the amino terminal amino acid to position 28 in the carboxyl terminus of VIP were synthesized. Synthesis was performed using standard solid phase methods. Homogeneity of the peptides was assessed by thin layer chromatography and analytical reverse phase HPLC, and purity was at least 97% for each peptide.

Using the same methods, one VIP analog with multiple alanine substitutions ([A][2,9,11,19,24,25,27,28]VIP) was also synthesized and purified. [Lys15,Arg16]Leu17VIP-1-7 (GRF8-27) (a VIP analog selective for VPAC1-R) (Harmar et al., 1998; Ito et al., 2000) and Ro 25-1553 (a cyclic VIP analog selective for VPAC2-R) (O’Donnell et al., 1994; Harmar et al., 1998; Ito et al., 2000) were also synthesized using a similar procedure.

Transfection of CHO Cells with Human VPAC1-R (hVPAC1-R) and Rat VPAC1-R (rVPAC1-R), and PANC1 Cells with rVPAC1-R and Selection of Stable Transfectants. Construction of the rVPAC1-R, hVPAC1-R expression vector was described previously (Ito et al., 2000, 2001). Construction of hVPAC1-R or rVPAC1-R stably transfected CHO or PANC1 cells (hVIP1-R or rVIP1-R/CHO or PANC1 cells) was described previously (Ito et al., 2001). To prepare stably transfected CHO and PANC1 cells containing hVPAC1-R or rVPAC1-R, after transfection with lipofectAMINE, individual colonies were isolated and expanded, and cloned cells were screened for VIP-R expression by receptor binding of [125I]-VIP. For rat and human VIP-Rs in each cell type at least four clones were isolated and binding of [125I]-VIP assessed in more detail by dose-inhibition curves for VIP and related peptides. For each cell type and species the affinities of the different clones were similar for VIP and the other peptides tested. The clone showing the highest binding was selected for additional studies.

Preparation of VIP. VIP was prepared by acid hydrolysis of purified human VIP (Bachem Biosciences, King of Prussia, PA) and purified by reverse phase HPLC and analytical reverse phase HPLC, and purity was at least 97% for each peptide. Using the same methods, one VIP analog with multiple alanine substitutions ([A][2,9,11,19,24,25,27,28]VIP) was also synthesized and purified. [Lys15,Arg16]Leu17VIP-1-7 (GRF8-27) (a VIP analog selective for VPAC1-R) (Harmar et al., 1998; Ito et al., 2000) and Ro 25-1553 (a cyclic VIP analog selective for VPAC2-R) (O’Donnell et al., 1994; Harmar et al., 1998; Ito et al., 2000) were also synthesized using a similar procedure.

Transfection of CHO Cells with Human VPAC1-R (hVPAC1-R) and Rat VPAC1-R (rVPAC1-R), and PANC1 Cells with rVPAC1-R and Selection of Stable Transfectants. Construction of the rVPAC1-R, hVPAC1-R expression vector was described previously (Ito et al., 2000, 2001). Construction of hVPAC1-R or rVPAC1-R stably transfected CHO or PANC1 cells (hVIP1-R or rVIP1-R/CHO or PANC1 cells) was described previously (Ito et al., 2001). To prepare stably transfected CHO and PANC1 cells containing hVPAC1-R or rVPAC1-R, after transfection with lipofectAMINE, individual colonies were isolated and expanded, and cloned cells were screened for VIP-R expression by receptor binding of [125I]-VIP. For rat and human VIP-Rs in each cell type at least four clones were isolated and binding of [125I]-VIP assessed in more detail by dose-inhibition curves for VIP and related peptides. For each cell type and species the affinities of the different clones were similar for VIP and the other peptides tested. The clone showing the highest binding was selected for additional studies.
**Cell Culture.** The hVIP1-R/CHO and rVIP1-R/CHO cells were grown in HAM medium supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) antibiotics, and 300 μg/ml G418. The rVIP1-R/PANC1 cells were grown in DMEM supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) antibiotics, and 300 μg/ml G418. The hVIP1-R/PANC1 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) antibiotics, and 200 μg/ml G418. T47D breast cancer cells were grown in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) antibiotics, and 1.4 μM bovine insulin. H508 human colon cancer cells and SupT1 human lymphoblastoma cells were grown in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum, 1 mM sodium pyruvate, and 1% (v/v) antibiotics. Cultures were maintained in incubators at 37°C in an atmosphere of 5% CO2 and 95% air.

**Southern Blot Analysis.** Total RNA from human VPAC1- or transfected PANC1 cells and CHO, T47D cells, H508 cells, SupT1 cells, and T98G cells were used to synthesize first-strand cDNA. The first-strand cDNA was synthesized using 1.0 μg of total RNA with the First-Strand cDNA synthesis kit (Invitrogen). The probe for the hVIP1-R (a 327-bp fragment) was generated from huRNA with the First-Strand cDNA. The medium was supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) antibiotics, and 300 μg/ml G418. T47D breast cancer cells were grown in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) antibiotics, and 1.4 μM bovine insulin. T98G human glioblastoma cells were grown in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum, 1 mM sodium pyruvate, and 1% (v/v) antibiotics. Cultures were maintained in incubators at 37°C in an atmosphere of 5% CO2 and 95% air.

**Preparations and Degradation Study of [125I]-[Ala2,8,9,11,19,24,25,27,28]VIP.** [125I]-[Ala2,8,9,11,19,24,25,27,28]VIP at a specific activity of 2200 Ci/mmol was prepared by a modification of the methods described previously (Zhou et al., 1989). [125I]-[Ala2,8,9,11,19,24,25,27,28]VIP or [125I]-VIP corresponding to 500,000 cpm (250 pM) was incubated in 1 ml of standard incubation solution in the absence of bacitracin, with or without VIP1-R/PANC1 cells (0.3 × 106 cells/ml) at 37°C for 7.5 min. After incubation, aliquots were centrifuged and distribution of the radiospecificity of 1 μM VIP. The IC50 was calculated using the curve-fitting program Kaleidagraph.

**Preparation of Dispersed Pancreatic Acini.** Dispersed acini from guinea pig and rat were prepared as described previously (Ito et al., 2000). Unless specified otherwise dispersed acini from the pancreas of one animal were suspended in 100 ml of standard incubation solution. All incubations were at 37°C. The incubation solution was equilibrated with 100% O2, and incubations for measurement of amylase release were performed with 100% O2 as the gas phase.

**Assessment of Amylase Release from Guinea Pig Pancreatic Acini.** Amylase release was measured using the procedure published previously (Ito et al., 2000). Amylase activity was determined using the Phadebas reagent, and results were expressed as the percentage of the total cellular amylase released into the extracellular medium during the incubation. For all peptides the EC50 was calculated, which was the concentration of the peptide that gave half-maximal stimulation of a maximally effective concentration of VIP (10 nM). The EC50 was calculated using the curve-fitting program Kaleidagraph (Synergy Software, Reading, PA).

**Binding Studies.** Binding of [125I]-VIP to pancreatic acini was performed as described previously (Zhou et al., 1989; Fishbein et al., 1994; Ito et al., 2000). Acini were incubated with nonradioiodinated peptides for 45 min at 37°C with 75 pM [125I]-VIP without (total binding) or with 1 μM VIP (nonsaturable binding). Samples (100 μl) of cell suspension were centrifuged through silicon oil (density = 1.05) in microcentrifuge tubes to separate bound from unbound ligand. Radioactivity was determined by a Packard autogamma counter (Packard Instrument Co., Meriden, CT). Nonsaturable binding for [125I]-VIP was less than 3% of total binding.

**Binding of [125I]-VIP to rVIP1-R/PANC1, rVIP1-R/CHO, hVIP1-R/CHO, hVIP1-R/PANC1, and T47D breast cancer cells** was performed by incubation in standard incubation solution containing 0.05% (w/v) bacitracin for 60 min at room temperature. The separations of bound from free radioactivity were obtained by centrifugation of cells through 2% (w/v) BSA in standard incubation solution. Nonsaturable binding for [125I]-VIP was less than 5% of total binding. For all peptides, the IC50 was calculated, which was the concentration that gave half-maximal inhibition of that seen with a saturating concentration of 1 μM VIP. The IC50 was calculated using the curve-fitting program Kaleidagraph.
ity in the supernatants was analyzed by HPLC. Each supernatant, including 125I-VIP corresponding to 220,000 cpm or 125I-[Ala2,9,11,19,24,25,27,28]VIP corresponding to 180,000 cpm, was injected onto an HPLC with a Vydac C18 column. Fractions (1 ml) were collected and radioactivity determined.

**Statistical Analysis.** The results are means ± S.E.M. of three or more experiments. IC_{50} and EC_{50} were calculated using the curve-fitting program Kaleidagraph. Binding curves for VIP_{1}-R/PANC1 cells and T47D cells were fitted using a least-squares curve fitting program, LIGAND (P. J. Munson; NIH, Bethesda, MD), to calculate dissociation constant (K_{d}) and binding capacities (B_{max}). Statistical comparisons were made using the Student’s t test.

**Results**

**Expression of Subtypes of VIP Receptors in Human Tumor Cell Lines.** To access the pharmacology of VIP at the VPAC_{1} receptor we used two different approaches. Because VIP can interact with VPAC_{2}-R also and at high concentrations with the PACAP receptor (Harmar et al., 1998) and because many cell types contain more than one subtype of VIP/PACAP receptor (Waschek et al., 1995; Reubi et al., 2000), we first prepared cell lines with VPAC_{1}-R stably transfected. Second, to confirm that the pharmacology in the transfected cell lines resembled the native receptor, we used cells that contained only or >90% native VPAC_{1}-R. To obtain a native hVPAC_{1}-R in sufficient numbers to determine pharmacology we screened a number of human cell lines reported to contain hVIP receptors (Fig. 1). The expression of human VIP receptors is reported in T47D human breast cancer cells (Waschek et al., 1995), NCI-H508 human colon cancer cells (Frucht et al., 1992), T98G human glioblastoma cells (Ver- tongen et al., 1996), and Sup T1 human lymphoblastoma cells (Robberecht et al., 1996). RT-PCR with Southern blotting with probes specific for hVPAC_{1}-R or hVPAC_{2}-R was performed to determine whether mRNA for these receptors was present in these cells (Fig. 1). T47D cells expressed hVPAC_{1}-R mRNA only (Fig. 1, lanes 3 and 13). H508 cells showed expression of both hVPAC_{1}-R and hVPAC_{2}-R mRNA (Fig. 1, lanes 2 and 12). T98G cells and Sup T1 cells expressed hVPAC_{2}-R mRNA only (Fig. 1, lanes 1, 6, 7, 14, and 15).

To confirm that T47D cells possessed only hVPAC_{1}-R, receptor binding studies with 125I-VIP were performed with dose-inhibition curves by using native VIP, [Lys^{15}, Arg^{16}, Leu^{27}]VIP(1–7)GRF(8–27) (a VIP analog selective for VPAC_{1}-R) (Harmar et al., 1998; Ito et al., 2000) and Ro 25-1553 (a cyclic VIP analog selective for VPAC_{2}-R) (ODonnell et al., 1994; Harmar et al., 1998; Ito et al., 2000). The IC_{50} of these peptides was 1.6 nM, 8.9 nM, and 1.6 μM, respectively (data not shown). The dose-inhibition curve of the VPAC_{2}-R ligand Ro 25-1553 was not biphasic as would be expected if high-affinity VPAC_{2}-R was present in sufficient numbers to be detected by binding studies (Ito et al., 2000). These results demonstrated T47D cells possessed only hVPAC_{1}-R by binding studies. A similar study on H508 human colon cancer cells demonstrated significantly lower levels of binding; therefore, T47D cells were used for all further studies.

**TABLE 1**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Human hVIP_{1}-R/CHO</th>
<th>Human hVIP_{1}-R/PANC1</th>
<th>Rat rVIP_{1}-R/CHO</th>
<th>Rat rVIP_{1}-R/PANC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>0.20 ± 0.01</td>
<td>1.7 ± 0.1</td>
<td>0.18 ± 0.01</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>[Ala^{1}]VIP</td>
<td>16.9 ± 0.7</td>
<td>89.1 ± 8.2</td>
<td>29.5 ± 1.1</td>
<td>309 ± 15</td>
</tr>
<tr>
<td>[Ala^{2}]VIP</td>
<td>0.43 ± 0.04</td>
<td>3.6 ± 0.5</td>
<td>0.17 ± 0.01</td>
<td>1.9 ± 0.1</td>
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<tr>
<td>[Ala^{3}]VIP</td>
<td>74.1 ± 9.4</td>
<td>182 ± 28</td>
<td>43.7 ± 3.7</td>
<td>148 ± 13</td>
</tr>
<tr>
<td>[Ala^{4}]VIP</td>
<td>2.2 ± 0.2</td>
<td>32.7 ± 3.4</td>
<td>4.2 ± 3.7</td>
<td>56.2 ± 5.1</td>
</tr>
<tr>
<td>[Ala^{5}]VIP</td>
<td>724 ± 91</td>
<td>1148 ± 155</td>
<td>6.0 ± 42</td>
<td>24 ± 277</td>
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<tr>
<td>[Ala^{6}]VIP</td>
<td>17.8 ± 0.7</td>
<td>51.3 ± 9.6</td>
<td>16.5 ± 0.8</td>
<td>53.7 ± 7.2</td>
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<tr>
<td>[Ala^{7}]VIP</td>
<td>0.17 ± 0.04</td>
<td>1.6 ± 0.3</td>
<td>0.19 ± 0.01</td>
<td>2.0 ± 0.1</td>
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<tr>
<td>[Ala^{8}]VIP</td>
<td>0.29 ± 0.03</td>
<td>1.8 ± 0.2</td>
<td>0.16 ± 0.01</td>
<td>0.49 ± 0.06</td>
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<tr>
<td>[Ala^{9}]VIP</td>
<td>61.7 ± 1.9</td>
<td>56.2 ± 4.1</td>
<td>15.9 ± 1.4</td>
<td>168 ± 14</td>
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<tr>
<td>[Ala^{10}]VIP</td>
<td>0.22 ± 0.02</td>
<td>2.8 ± 0.5</td>
<td>0.34 ± 0.05</td>
<td>0.42 ± 0.06</td>
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<tr>
<td>[Ala^{11}]VIP</td>
<td>53.7 ± 1.5</td>
<td>61.7 ± 4.7</td>
<td>12.3 ± 1.2</td>
<td>30.0 ± 2.7</td>
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<tr>
<td>[Ala^{12}]VIP</td>
<td>6.6 ± 0.1</td>
<td>7.2 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>10.0 ± 0.4</td>
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<tr>
<td>[Ala^{13}]VIP</td>
<td>24.6 ± 1.2</td>
<td>174 ± 11</td>
<td>30.0 ± 3.6</td>
<td>51.3 ± 5.0</td>
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<td>[Ala^{14}]VIP</td>
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<td>16.2 ± 2.8</td>
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<td>[Ala^{15}]VIP</td>
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<td>6.6 ± 0.6</td>
<td>0.93 ± 0.04</td>
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<td>[Ala^{16}]VIP</td>
<td>0.74 ± 0.07</td>
<td>4.5 ± 1.1</td>
<td>1.6 ± 0.1</td>
<td>3.6 ± 0.4</td>
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<tr>
<td>[Ala^{17}]VIP</td>
<td>0.59 ± 0.03</td>
<td>4.1 ± 0.5</td>
<td>0.85 ± 0.06</td>
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<td>[Ala^{18}]VIP</td>
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<td>13.1 ± 0.8</td>
<td>0.38 ± 0.03</td>
<td>0.51 ± 0.12</td>
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<td>[Ala^{19}]VIP</td>
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<td>5.0 ± 0.3</td>
<td>0.75 ± 0.06</td>
<td>1.0 ± 0.1</td>
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<td>[Ala^{20}]VIP</td>
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<td>7.6 ± 0.9</td>
<td>11.1 ± 0.3</td>
<td>2.8 ± 0.2</td>
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<tr>
<td>[Ala^{21}]VIP</td>
<td>26.9 ± 1.8</td>
<td>170 ± 21</td>
<td>33.9 ± 2.6</td>
<td>204 ± 0.6</td>
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<tr>
<td>[Ala^{22}]VIP</td>
<td>0.24 ± 0.02</td>
<td>3.2 ± 0.3</td>
<td>0.26 ± 0.01</td>
<td>1.3 ± 0.2</td>
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<tr>
<td>[Ala^{23}]VIP</td>
<td>0.49 ± 0.06</td>
<td>3.4 ± 0.3</td>
<td>0.27 ± 0.01</td>
<td>0.47 ± 0.05</td>
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<tr>
<td>[Ala^{24}]VIP</td>
<td>2.6 ± 0.1</td>
<td>11.2 ± 1.6</td>
<td>1.7 ± 0.1</td>
<td>4.9 ± 0.1</td>
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<tr>
<td>[Ala^{25}]VIP</td>
<td>0.57 ± 0.02</td>
<td>2.3 ± 0.2</td>
<td>0.59 ± 0.08</td>
<td>2.0 ± 0.2</td>
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<tr>
<td>[Ala^{26}]VIP</td>
<td>0.54 ± 0.04</td>
<td>2.7 ± 0.2</td>
<td>0.20 ± 0.01</td>
<td>1.1 ± 0.1</td>
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</tbody>
</table>

6 T47D breast cancer cells possess native hVPAC_{1}-R only, whereas in the CHO and PANC1 cells either hVPAC_{1}-R or hVPAC_{2}-R was stably transfected. The indicated cell type was incubated with 75 pM 125I-VIP and various concentrations of the unlabeled alanine-substituted VIP analog. The concentration of peptide causing half-maximal inhibition (IC_{50}) of 125I-VIP binding was that concentration causing half-maximal inhibition of the saturable binding caused by 1 μM VIP calculated using the curve-fitting program Kaleidagraph. In each experiment, each value was determined in duplicate and values given are means ± S.E.M. from at least three separate experiments.

7 Native VIP has an alanine in position 4 and 18.
To characterize the native hVPAC1-R further, a dose-inhibition curve by VIP of $^{125}$I-VIP binding was performed. VIP caused half-maximal inhibition at $1.6 \pm 0.1$ nM (Table 1). Analysis of the VIP dose-inhibition curve by a curve-fitting program demonstrated it was significantly better ($p = 0.007$) fit by a two-binding site model ($n = 4$) consistent with the Hill

Fig. 2. Relative affinities of various single alanine-substituted VIP analogs for native or transfected human (top), rat or guinea pig (bottom) VPAC1 receptors compared with native VIP. Top, hVPAC1-R-transfected CHO and PANC1 cells and T47D breast cancer cells, which natively possess hVPAC1-R, were incubated for 60 min at room temperature with 75 pM $^{125}$I-VIP alone or with various concentrations (0.01 nM–100 nM) of the indicated alanine-substituted VIP analogs or native VIP. By using the curve-fitting program Kaleidagraph, the IC$_{50}$ for all peptides was calculated, which was the concentration that gave half-maximal inhibition of the saturable binding seen with 75 pM $^{125}$I-VIP alone. Results are shown with VIP analogs with a single alanine substitution from position 1 on the left to position 28 of VIP on the right. Native VIP has an alanine in position 4 and 18. Data are expressed as the ratio of the affinity of a given peptide divided by the affinity (IC$_{50}$) of native VIP. The affinity of native VIP for human VPAC1-transfected cells or T47D breast cancer cells was as follows: hVIP1-R/CHO cells, $0.2 \pm 0.01$ nM; hVIP1-R/PANC1 cells, $1.7 \pm 0.1$ nM; and T47D cells, $1.6 \pm 0.1$ nM. Bottom, rat VPAC1 receptor-transfected PANC1 cells were incubated for 60 min at room temperature with 75 pM $^{125}$I-VIP alone or with various concentrations (0.01 nM–100 nM) of the indicated alanine-substituted VIP analogs or native VIP. Rat or guinea pig acini, which possess primarily VPAC1 (i.e., 90%) (Jensen, 1994; Ito et al., 2000), was incubated for 45 min at 37°C under similar conditions described above. The affinity (IC$_{50}$) of native VIP for rat VPAC1-R-transfected PANC1 cells, or rat or guinea pig acini was as follows: rVIP1-R/PANC1 cells, $0.54 \pm 0.03$ nM; rat acini, $2.4 \pm 0.2$ nM; and guinea pig acini, $2.5 \pm 0.1$ nM. Less or more potency indicates the amount of decrease or the increase in affinity a given alanine substitution caused compared with native VIP, respectively. Data are the means from at least three experiments, and in each experiment each point was determined in duplicate.
better understand the pharmacophore of VIP for the VPAC₁ receptor, we performed an alanine scan by the substitution of each amino acid one at a time with alanine. We analyzed both the human VPAC₁-R (by using hVPAC₁-R transfected CHO cells, and T47D cells containing native hVPAC₁-R) as well as rVPAC₁-R (by using rat pancreatic acini containing native rVPAC₁-R and rVPAC₁-R-transfected PANC1 cells), as well as guinea pig pancreatic acini possessing native VPAC₁-R. A recent study has demonstrated rat and guinea pig acini possess primarily VPAC₁-R, as well as VPAC₁-R as the VPAC₁-R-transfected PANC1 cells. A recent study has demonstrated rat and guinea pig acini possess primarily VPAC₁-R (Jiang et al., 1997). hVPAC₁-R-transfected PANC1 cells were used because the PANC1 cells we used did not possess natively VPAC₁-R, confirmed by RT-PCR with Southern blotting and binding studies (data not shown).

Affinity of Single Alanine-Substituted Analogs of VIP for Human, Rat, or Guinea Pig VPAC₁ Receptor. To better understand the pharmacophore of VIP for the VPAC₁ receptor, we performed an alanine scan by the substitution of each amino acid one at a time with alanine. We analyzed both the human VPAC₁-R (by using hVPAC₁-R transfected CHO cells, and T47D cells containing native hVPAC₁-R) as well as rVPAC₁-R (by using rat pancreatic acini containing native rVPAC₁-R and rVPAC₁-R-transfected PANC1 cells), as well as guinea pig pancreatic acini possessing native VPAC₁-R. A recent study has demonstrated rat and guinea pig acini possess primarily VPAC₁-R (Jiang et al., 1997). hVPAC₁-R-transfected PANC1 cells were used because the PANC1 cells we used did not possess natively VPAC₁-R, confirmed by RT-PCR with Southern blotting and binding studies (data not shown).

In general, the alanine scan results were similar in terms of relative affinities to VIP with each of the cell lines containing hVPAC₁-R (Fig. 2; Table 1). In terms of absolute affinities the results were almost identical for each alanine-substituted VIP analog with T47D cells and hVIP₁-R/PANC1 (Table 1). However, for 23 of the 26 alanine-substituted analogs the affinity varied from 1.1- to 121-fold with a mean of 5.9-fold greater with hVIP₁-R/CHO cells than T47D and 1.1- to 14.9-fold with a mean of 7.4-fold greater with hVIP₁-R/CHO cells than hVIP₁-R/PANC1 cells (Table 1; Fig. 3). As is evident from Fig. 3, top, there was an excellent correlation (r = 0.97; p < 0.00001) between the IC₅₀ values obtained from each single alanine-substituted analog for the native hVPAC₁-R on T47D cells and hVPAC₁-R-transfected PANC1 cells. The correlation between the IC₅₀ for each analog between the native hVPAC₁-R and hVIP₁-R/PANC1 cells (r = 0.50) (Fig. 3, bottom) was significantly (p < 0.01) lower than that obtained for the hVPAC₁-R-transfected PANC1 cells. When a similar analysis was performed with relative IC₅₀ values compared with VIP, the correlation coefficient between the native hVPAC₁-R in T47D cells and hVIP₁-R/PANC1 cells (r = 0.97) was significantly better than that between native hVPAC₁-R cells and hVIP₁-R/CHO cells (r = 0.47). Furthermore, the slope of the regression equation was close to unity (i.e., 1.3×) with native and hVIP₁-R/PANC1 cells, whereas it was greater than unity with native and hVIP₁-R/CHO cells (i.e., 2.8×). Replacement of Phe⁶ (500-fold decrease), Leu²⁹ (100-fold decrease), Arg¹⁴ (70- to 120-fold decrease), and Asp³ (80- to 370-fold decrease) resulted in VIP analogs with the greatest decrease in affinity (Fig. 2; Table 1), suggesting these amino acids were the most
important for high-affinity interaction at the hVPAC1-R. In 12 of the 26 alanine-substituted analogs either no change or a decrease in affinity <4-fold occurred (Fig. 2). These included alanine substitution for Ser2, Asp8, Asn9, Thr11, Gln16, Met17, Val19, Tyr22, Ser25, Leu27, and Asn28 (Fig. 2; Table 1).

Because guinea pigs or rats are frequently used for in vivo studies and it is reported the VIP receptor pharmacophore is the same for human and guinea pig (O’Donnell et al., 1991; Bolin et al., 1995), we also wanted to determine whether the native and stably transfected VPAC1-R in these species has a similar pharmacophore to that seen with the hVPAC1-R cells. In general, there was a close agreement between the relative affinities of the various VIP-alaminated analogs for each of the four rat and guinea pig VPAC1-R-containing cells (Fig. 2; Tables 1 and 2). However, similar to the hVPAC1-R, almost all of the 26 alanine-substituted VIP analogs had a 1.2- to 42-fold greater affinity, with a mean of 6.6-fold for rVPAC1-R-transfected CHO cells over rVPAC1-R-transfected PANC1 cells or native rVPAC1-R in rat pancreatic acini (Tables 1 and 2). Similar to the human VPAC1-R, with the rat and guinea pig VIP-VPAC1-R, substitution of alanine for His5, Asp8, Phe6, Thr7, Tyr10, Arg12, Arg14, and Leu23 had a marked effect on potency, decreasing affinity >15-fold (Fig. 2; Tables 1 and 2). Furthermore, similar to the human VPAC1-R, the rat and the guinea pig VPAC1-Rs were most affected by alanine substitution for Phe6 (>1000-fold decrease), Leu23 (>90-fold decrease), or Asp8 replacement (>100-fold decrease), and alanine substitution for Asp8 and Asn9 increased affinity over that seen with VIP (Fig. 2; Tables 1 and 2). With the rat and guinea pig VPAC1-R, there was no change in affinity seen by alanine substitution for Lys15, which was significantly different (p < 0.001) from the 10-fold decrease in affinity seen at the hVPAC1-R with this change (Fig. 2; Tables 1 and 2). Another difference in the pharmacophore between human and rat or guinea pig VPAC1-R was the effect of alanine substitution for Lys21. In human VPAC1-R cells alanine substitution in this position caused a 3-fold decrease in affinity, whereas in rat and guinea pig VPAC1-R, the change was significantly different (p = 0.03) with no change in affinity seen (Fig. 2; Tables 1 and 2). These results from alanine scanning demonstrate that in general the rat and guinea pig VPAC1-Rs have a similar pharmacophore to human VPAC1-R. However, there are important differences, particularly in the importance of the side chains of the substitutions in position 15 and 21.

Affinity of Single d-Amino Acid-Substituted Analogs of VIP for Human, Rat, or Guinea Pig VPAC1 Receptor.

To investigate the importance of the orientation of the amino acid backbone substitution at each position of VIP for determining affinity at the human VPAC1-R, similar studies to those performed with alanine-substituted analogs were performed with each amino acid of VIP replaced one at a time with its d-isomer (Fig. 4; Table 3). As seen in alanine scanning, the VIP pharmacophore demonstrated by comparing the relative affinity of each d-amino acid-substituted analog

<table>
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<th>Peptide</th>
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<th>Rat Acini</th>
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<td>Binding of 125I-VIP (IC&lt;sub&gt;50&lt;/sub&gt;)</td>
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<tr>
<td>VIP&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>[Ala&lt;sup&gt;22&lt;/sup&gt;]VIP</td>
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<td>8.9 ± 0.5</td>
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<td>0.059 ± 0.022</td>
<td>4.3 ± 0.2</td>
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<td>[Ala&lt;sup&gt;26&lt;/sup&gt;]VIP</td>
<td>0.051 ± 0.009</td>
<td>4.3 ± 0.4</td>
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</table>

* Native VIP has an alanine in position 4 and 18.
to VIP was generally close among different VPAC₁-R cell types (Fig. 4). The native hVPAC₁-R on T47D cells had almost identical affinities for the 28 different analogs to hVPAC₁-R-transfected PANC1 cells (Table 3). In contrast, with D-amino acid substitution in most positions, VPAC₁-R transfected into CHO cells had a higher affinity, varying from 1.3- to 18.6-fold with a mean of 6.5-fold for the identical D-amino acid-substituted analog compared with the results from the other two cell lines (Fig. 4; Table 3). Substitution of D-His¹, D-Gln¹⁶, and D-Leu²⁷ had the least effect, causing only a 2- to 3-fold decrease in affinity compared with VIP. The greatest decrease occurred with substitution of D-Phe⁶ (>80-fold), D-Thr⁷ (>130-fold), D-Asp⁸ (>80-fold), D-Thr¹¹ (>50-fold), D-Lys²¹ (>60-fold), and D-Tyr²² (>50-fold), demonstrating the orientation of these amino acids’ side chains were particularly important for high-affinity receptor interaction.

D-Amino acid scans of VIP were also performed with rat and guinea pig VPAC₁-Rs (Fig. 4; Tables 3 and 4). In general, a similar pharmacophore to that obtained on the hVPAC₁-R was seen, with the side chain amino acid orientation of Phe⁶, Thr⁷, and Asp⁸ being particularly important and that of Asn⁹ and Leu²⁷ not being important for high-affinity interaction (Fig. 4; Tables 3 and 4). Human VPAC₁-R differed from rat and guinea pig VPAC₁-R in the importance of the side chain orientation of His¹ and Tyr²². In human VPAC₁-R, substitution of a D-amino acid for His¹ caused a minimal decrease in affinity (no change or <3-fold), whereas in rat and guinea pig VPAC₁-R it caused a 5- to 10-fold decrease. Conversely, in rat and guinea pig VPAC₁-R, substitution of a D-Tyr²² caused <15-fold change in affinity, whereas in the human VPAC₁-R, these substitutions caused a 70-fold decrease. These results demonstrate that the pharmacophore revealed by D-amino acids in the human VPAC₁-R is primarily determined by the side chain orientation of the amino acids.

Fig. 4. Relative potencies of D-amino acid-substituted VIP analogs for native or transfected human (top), rat, or guinea pig (bottom) VPAC₁ receptors compared with native VIP. The experimental conditions were the same as described in the legend to Fig. 2. The IC₅₀ for each peptide was calculated as described in the legend to Fig. 2, and the data are expressed as the ratio of the affinity of a given peptide to that of native VIP as described in the legend to Fig. 2. Data are the means from at least three experiments and in each experiment each point was determined in duplicate.
Acid scanning between human VPAC1-R and rat or guinea pig VPAC1-R has important differences.

**Affinity and Biological Activity of Single Alanine-Substituted Analogs of VIP in Guinea Pig Acini.** To compare the effect of alanine substitutions in VIP on biological activity with its effect on the affinity for the hVPAC1-R, we used guinea pig pancreatic acini, because studies (Ito et al., 2000) demonstrate that 90% of the amylase release through the VIP receptor in this species is mediated by VPAC1-R. At a concentration of 1 mM, all of the 26 single alanine-substituted analogs had full agonist activity, causing a 4-fold increase in enzyme secretion, which was equal in efficacy to a maximally effective concentration of VIP (10 nM) (data not shown). Alanine substitution for His1, Asp3, Phe6, Thr7, Tyr10, Arg12, Arg14, and Leu27 produced a marked decrease of greater than 20-fold in affinity for either binding affinity or potency for stimulating amylase release (Fig. 5; Table 2), especially Asp3, Phe6, and Leu27. In contrast, substitutions for Thr11, Lys15, Asn24, or Leu27 increased the potency for stimulating amylase secretion (Fig. 5; Table 2). There was a very close correlation (r = 0.997; p < 0.0001) between the ability of each alanine-substituted analog to stimulate enzyme secretion from pancreatic acini and inhibit 125I-VIP binding to VPAC1-R on pancreatic acini. These results demonstrate that the replacement of the various amino acid side chains of VIP by a methyl group did not result in a dissociation between binding affinity and potency for receptor activation, demonstrating that for all of the alanine-substituted analogs, binding affinity and receptor activation are closely coupled.

**Affinity and Biological Activity of the d-Amino Acid-Substituted Analog of VIP in Guinea Pig Pancreatic Acini.** Each of the 28 d-amino acid VIP analogs was an agonist for stimulating amylase release (Fig. 5; Table 4). If a sufficiently high concentration was used, all but [D-Phe6]VIP was a full agonist. [D-Phe6]VIP was a partial agonist causing 50 ± 3% of the maximal secretion caused by a maximally effective concentration of VIP. There was a close correlation (r = 0.85; p < 0.0001) between the binding affinity of each d-amino acid-substituted analog and its potency for stimulating amylase release (Fig. 5; Table 4). The stereochemistry of Asn9, Glu16, Ala18, Lys20, and Leu27 had either no or a minimal (<3-fold change) effect on biological potency. In contrast, the stereochemistry of Phe6, Thr7, Asp3, and Lys21 was essential to high potency for secretion as it was for binding affinity (Fig. 5; Table 4).

**Affinity, Biological Activity, and Stability of a Simplified VIP Analog with Multiple Alanine Substitution.** In an attempt to identify a simplified human VPAC1-R ligand, we used the alanine and d-amino acid scan data from our study to identify amino acids where the replacement of a given amino acid had either no or minimal effect on receptor affinity to design a simplified analog. The analog, [Ala2,8,9,11,19,24,25,27,28]VIP, had an equal or better affinity

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**TABLE 3**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Human</th>
<th>Rat</th>
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<tbody>
<tr>
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<td>hVIP1-R/CHO</td>
<td>hVIP1-R/PANC1</td>
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<tr>
<td>[Ala2,8,9,11,19,24,25,27,28]VIP</td>
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</table>

* T47D breast cancer cells possess native hVPAC1-R only, whereas in the CHO and PANC1 cells either hVPAC1-R or rVPAC1-R was stably transfected. 125I-VIP binding was performed as described in Table 1. The concentration of peptide causing half-maximal inhibition (IC50) of 125I-VIP binding was that concentration causing half-maximal inhibition of the saturable binding caused by 1 μM VIP calculated using the curve-fitting program Kaleidagraph. In each experiment, each value was determined in duplicate, and values given are means ± S.E.M. from at least three separate experiments.
Alanine scanning demonstrated the side chain substitutions that are most important for determining the overall pharmacophore of VIP for the human VPAC₁ receptors. Phe⁶, Arg¹⁴, and Leu²³, which were also found to be important in our study. The difference from our results is likely explained by the fact that the VIP pharmacophore for the native human VPAC₁ receptor, alanine substitution of Lys¹⁵, Gln¹⁶, Lys²⁰ caused little or no change. These results have both similarities and differences from previous studies. In one (O’Donnell et al., 1991) of the two studies (O’Donnell et al., 1991; Nicole et al., 2000) assessing the VIP pharmacophore at human VIP receptors in which binding to human lung membranes was assessed, it was concluded using alanine scanning that the most important amino acids in determining high-affinity interaction were Thr⁷, Tyr¹⁰, and Tyr²² in addition to Asp³, Phe⁶, Arg¹⁴, and Leu²³, which were also found to be important in our study. The difference from our results is likely explained by the fact that the lung contains both VPAC₁ and VPAC₂ receptors (Usdin et al., 1994; Busto et al., 2000). This conclusion is supported by recent studies that demonstrate Thr⁷, Tyr¹⁰, and Tyr²² are much more important in determining the affinity of VIP for the VPAC₂ receptor than the VPAC₁ receptor (Nicole et al., 2000). Our results are in agreement with a recent study (Nicole et al., 2000) with human VPAC₁-R-transfected CHO cells, which concluded from alanine scanning that His¹, Phe⁶, Arg¹⁴, and Leu²³ were very important in determining the VIP pharmacophore for the human VPAC₁ receptor. However, our results on the relative importance of these different amino acids as well as those having an intermediate or no effect on affinity differ significantly from Nicole et al. (2000). That this difference was likely due to the different cell expression systems used was supported by the results in our study. We found that the VIP pharmacophore for either rat or human VPAC₁ receptors when expressed in CHO cells, but not PANC1 cells, differed significantly from that determined using cells possessing native VPAC₁ receptors. Our results using T47D cells and hVPAC₁-transfected PANC1 cells demonstrated Ser⁵, Asp⁵, Asn⁹, Thr¹¹, Val¹⁹, Asn²⁴, Ser²⁵, Leu²⁷, and Asn²⁸ substitutions with alanine caused a ≥2-fold decrease in affinity in either of these cells and these amino acid substitutions caused little or no loss of agonist potency. When these nine amino acids were alanine-substituted in VIP to form a simplified VIP analog, there was no loss of potency for VPAC₁ in any species (Fig. 6). [Ala³,⁹,¹¹,¹⁹,2⁴,2⁵,2⁷,2⁸]VIP also had a greater metabolic stability, demonstrating no degradation by VPAC₁-R alone, thereby suggesting it could be useful in vivo.

The present study for the first time provides insight into the VIP pharmacophore for a human VIP receptor by using D-amino acid scanning. This approach can provide informa-
tion on the importance of the orientation of a given amino acid side chain (Bolin et al., 1995; Coy, 2000). Such a strategy has been successfully used to investigate the pharmacophore and design potent agonists of growth hormone-releasing factor, which shares a close homology to VIP (Coy, 2000). This approach demonstrated that, except for substitution of D-His¹, D-Gln¹⁶, and D-Leu²⁷, alteration of the orientation of the side chain of any of the other 25 amino acids in VIP had a significant effect on receptor affinity in all species. The largest effects were seen with substitution of D-amino acids in the NH₂-terminal amino acids Phe⁶, Thr⁷, Asp⁸, and in the COOH-terminal amino acids Val¹⁹, Lys²¹, Tyr²², and Ile²⁶. Circular dichroism and nuclear magnetic resonance data obtained in organic solvents indicate VIP has a helical conformation, especially involving residues 15–28 and some studies suggest the N terminus has either one or two type-III β-bends at residues 2–5 and 7–10 (Fournier et al., 1988; Musso et al., 1988; Fry et al., 1989; Wray et al., 1998) (Fig. 7). In one study in 50% methanol/water (Fry et al., 1989), the side chains of Asp³, Phe⁶, Thr⁷ were found to be clustered near the NH₂ terminus and a second cluster of residues involving Ala¹⁸, Val¹⁹, Tyr²², Ile²⁶ occur near the COOH terminus. It was proposed (Fry et al., 1989) that hydrogen bonding between Thr⁷ and Asp³ and Asp⁸ and Lys¹² may be important in stabilizing this helical structure. Based on these conformation studies, it was suggested VIP may have two separate regions that interact with the VIP receptor (Fry et al., 1989). Our data support this conclusion by both alanine
scanning and D-amino acid substitutions. We could find a clustering of NH2-terminal amino acids (residues 3, 6–8) and COOH-terminal amino acids (residues 19, 21, 23, 26) whose side chain conformation and backbone integrity are particularly important to the VIP pharmacophore. A decrease in affinity with alanine or D-amino acid substitution could be due to either altered binding where the structural change was important in ligand-VPAC1 receptor interaction or to the amino acid change altering the global structure of VIP, perhaps by altering its secondary structure. Our results suggest that differences in the affinities of VIP analogs with alanine substitutions for Leu13, Lys 15, Gln 16, Met17, Lys20, Lys21, Tyr22, Asn24, Ser25, Ile26, and Asn28 are not due to global conformation changes in VIP, but are likely due to differences in the importance of these amino acids' backbone substitution for receptor-ligand interaction in the different species. This speculation is supported by the fact that each of these single alanine-VIP analogs had unaltered receptor affinity in at least one assay system. With alanine substitutions for His1, Asp3, Val5, Phe6, Thr7, Tyr10, Arg12, Arg14, and Leu23, which had decreased receptor affinity in all systems examined, either mechanism discussed above could account for the decrease in affinity.

To determine the effect of amino acid substitutions in VIP on the VIP pharmacophore as assessed by its ability to activate the VIP receptor, we determined their ability to stimulate enzyme secretion from guinea pig pancreatic acini, which natively possess VPAC1 receptors (Ito et al., 2000). These cells possess no secretin or PACAP receptors (PAC 1) coupled to enzyme secretion (Jensen, 1994) that VIP could also interact with to stimulate secretion, and 90% of the secretion caused by activation of VIP receptors on these cells is due to VPAC1 receptors (Ito et al., 2000). In general, the VIP analog potencies for stimulating secretion correlated...
closely \((r > 0.85; \ p < 0.0001)\) with their relative binding affinities. All alanine- and \(\alpha\)-amino acid-substituted analogs were full agonists at the VPAC\(_1\) receptor except for \([\alpha\text{-Phe}^6]\text{VIP}\), which was a partial agonist. In previous studies \([\alpha\text{-Phe}^6]\text{VIP}\) or related analogs are reported to function as VPAC\(_1\) receptor antagonists (Jensen, 1994; Usdin et al., 1994; Gaudin et al., 1996), as a partial agonist (Fishbein et al., 1994), or as a full agonist (Bolin et al., 1995). In our study, \([\alpha\text{-Phe}^6]\text{VIP}\) functioned as a partial agonist of the VPAC\(_1\) receptor with 50% of the efficacy of VIP. However, because of its low affinity (IC\(_{50} > 0.1 \ \mu M\)) this analog is unlikely to be a good template for design of more potent antagonists. Our results are consistent with studies of the ability of these substituted VIP analogs to stimulate cyclic AMP accumulation in hVPAC\(_1\)-transfected CHO cells (Nicole et al., 2000) or to cause VIP receptor activation, resulting in the relaxation of guinea pig tracheal smooth muscle (O’Donnell et al., 1991; Bolin et al., 1995). These results demonstrate that a strategy of substitution of \(\alpha\)-amino acids in VIP is unlikely to yield potent antagonists.

In conclusion, we studied the VIP pharmacophore for the VPAC\(_1\) receptor in guinea pig, rat, and human by using both transfected cell systems and cells bearing native receptors. Our results demonstrated that there were significant species differences in the VIP pharmacophore for the VPAC\(_1\) receptor in these different species. Our studies also demonstrate...
that the type of cell used for the stable expression of the VPAC₁-R could have a significant effect on the VIP pharmacophore, a factor that has not been considered in a number of previous studies on VIP receptor pharmacology. Last, our results allowed us to synthesize a simplified VIP analog containing 11 alanines that had equal affinity to VIP for the VPAC₁-R in all species but was more metabolically stable than VIP. This analog should be useful as a prototype for design of selective VPAC₁ receptor agonists/antagonists that could be useful in vivo, either therapeutically or to elucidate VIP’s role in biological processes.

References


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