A Lymphocyte-Generated Fragment of Vasoactive Intestinal Peptide with VPAC1 Agonist Activity and VPAC2 Antagonist Effects

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ABSTRACT

Vasoactive intestinal peptide receptors 1 (VPAC1) and 2 (VPAC2) have been identified in humans. Cell lines expressing only VPAC1 (HT-29) or VPAC2 (Molt-4b) were identified using real-time reverse transcriptase polymerase chain reaction. Vasoactive intestinal peptide (VIP) and related peptides, VIP4–28, and VIP10–28, previously isolated from cultures of human leukocytes, were evaluated for their ability to bind to VPAC1 and VPAC2 and to increase the levels of cAMP in HT-29 and Molt-4b cells. VIP bound to membranes of HT-29 colon carcinoma cells and Molt-4b lymphoblasts with high affinity (K_D = 1.6 ± 0.2 and 1.7 ± 0.9 nM, respectively). VIP4–28 also demonstrated high-affinity binding (K_D = 1.7 ± 0.2 and 1.7 ± 0.7 nM in HT-29 and Molt-4b, respectively). VIP and VIP4–28 are potent VPAC1 agonists, inducing maximal 200- and 400-fold increases in cAMP, respectively. VIP demonstrated weak VPAC2 agonist activity, inducing a maximal 14-fold increase in cAMP. VIP4–28 had no VPAC2 agonist activity but demonstrated potent VPAC2 antagonist activity. VIP4–28 inhibited VPAC2-mediated increases in cAMP in Molt-4b cells up to 95%, but had no antagonistic effect on VPAC1. Lymphoblasts did not hydrolyze VIP4–28 to a form with VPAC1 antagonist activity. VIP4–28 thus is a lymphocyte-generated VIP fragment with potent agonist activity for VPAC1 and potent antagonist activity for VPAC2.

Several neuroendocrine peptides, originally thought to be synthesized exclusively in nerve cells and to interact only with those tissues innervated by peptidergic neurons have been demonstrated to modulate the synthesis of cytokines and growth factors. Neutrophils, eosinophils, and basophils have been shown to synthesize peptides, including somatostatin and VIP (Goetzl et al., 1985; Goetzl et al., 1988; O’Dorisio et al., 1980), whereas monocytes and lymphocytes express high-affinity receptors for these and other peptides (Danek et al., 1983; Ottaway et al., 1983; Scicchitano et al., 1987; O’Dorisio et al., 1989; Sreedharan et al., 1989). The structure and processing of prepro-VIP seems to differ in leukocytes compared with nerve cells; leukocytes produce and secrete both truncated and N-terminally extended peptides such as VIP10–28 and VIP6–28 (Hayakawa et al., 1984; Goetzl et al., 1988). Several VIP fragments, of which VIP4–28 is the most prominent, are generated from VIP by protease activity at the surface of lymphocytes (Goetzl et al., 1988). These peptides may be released into tissue fluids at nanomolar concentrations and therefore potentially exert significant immunoregulatory and other physiological effects. This study was designed to compare VPAC1 and VPAC2 recognition of VIP and several VIP variant peptides (Goetzl et al., 1988). Human cell lines that express only a single VIP receptor were identified by real-time RT-PCR; the relative affinities of these peptides for the two VIP receptors, VPAC1 in HT-29 colonic epithelial cells and VPAC2 in lymphoblastoid cells, were determined by competitive binding. Further experiments then examined the ability of VIP and VIP-related peptides to activate the cAMP signal transduction pathway in each cell line alone or in the presence of VIP.

Materials and Methods

Cell Culture. Molt-4b lymphoblasts (American Type Culture Collection, Manassas, VA) were grown in suspension cultures in 75-cm² flasks (Corning, Palo Alto, CA) in culture media consisting of RPMI 1640 medium with 15% heat-inactivated fetal bovine serum supplemented to final concentrations of 4 mM l-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. HT-29 colon carcinoma cells

ABBREVIATIONS: VIP, vasoactive intestinal peptide; RT-PCR, reverse transcriptase-polymerase chain reaction; PCR, polymerase chain reaction; TAMRA, 6-carboxytetramethylrhodamine; TCA, trichloroacetic acid; HPLC, high-pressure liquid chromatography; PAC1, pituitary adenylate cyclase-activating peptide receptor 1.
(American Type Culture Collection) were cultured as monolayers in 75-cm² flasks (Corning) in culture medium consisting of RPMI 1640 medium with 10% heat inactivated fetal bovine serum supplemented as described above. All media components were purchased from Invitrogen (Carlsbad, CA). Cells were incubated at 37°C in 5% CO₂ and grown to >90% confluence for membrane harvest or for signal transduction studies. Before use, cells were washed twice with Seligman’s balanced salt solution (Invitrogen). Cell number was evaluated by means of a Coulter counter. Cell viability was determined by trypan blue exclusion.

**Real-time RT-PCR.** Real-time RT-PCR was performed using the 5'-3’ nucleic activity of Taq polymerase to allow direct detection of the product by the release of a fluorescent reporter dye from a specific fluorescent-labeled probe during the PCR reaction. The probe consists of an oligonucleotide with a 5'-reporter dye (6-carboxyfluorescein) or VIC dye and 3'-quencher dye 6-carboxytrimethylammonium (TAMRA) (Applied Biosystems, Foster City, CA). Specific primers were designed for real-time RT-PCR of VPAC1, 5'-AGG CAG CGA GTT TGG AT-3' and 5'-GTG CAG AGC TTC CTG AAC-3'; VPAC2, 5'-CGT GAA CAG CAC TAG CAT CCA AGA AT-3' and 5'-GGT GAC GCT GTC CTC TCC CAC AT-3'; and rRNA, 5'-CGT GCTACACATCGCAAGA-3' and 5'-GCTGGAATTACCGCCGGCT-3'. The probes for VPAC1, CCACCTCCTGTGCGCCACGAT-TAMRA and VPAC2, AACAACAGGCTGACCTTGCTG-TAMRA were labeled with 6-carboxyfluorescein. The rRNA probe TGTTGACAGCAGATTGCCCTC-TAMRA was labeled with VIC, which has a different spectrum than the reporter dye of the VPAC1 and VPAC2 probes. The rRNA was used to ensure quality of RNA preparation, to account for efficiency of the reverse transcription, and to control for any loading variation of the initial cDNA amount. One standard curve for the target gene (VPAC1 or VPAC2), and one standard curve for the rRNA gene were generated for quantification. The rRNA standard curve was generated with known (picogram) amounts of a cloned rRNA gene (Lara-Marquez et al., 2001) to determine the expression of rRNA in each sample. Serial dilutions of linearized plasmids containing the human VPAC1 or VPAC2 gene were used to construct a standard curve of copy number versus threshold cycle (Ct). The actual number of copies of each target gene is thus extrapolated from each standard curve. VPAC1 and VPAC2 are measured as copy number and rRNA as picograms. The copy numbers are normalized against 100 pg of rRNA. The results are expressed as copy number per 100 pg of rRNA using the following formula: copy number/100 pg of rRNA = copy number of VPAC1 or VPAC2 x 100/100 pg of rRNA. One standard curve for the target gene (VPAC1 or VPAC2), and one standard curve for the rRNA gene were generated for quantification.

**Concentration Dependence of Antagonism by VIP4–28.** cAMP dose-response curves for VIP were generated in Molt-4b cells in the presence of various concentrations of VIP4–28 (0, 0.1 nM, 10 nM, and 1 µM), which were added simultaneously. The data were then compiled using GraphPad software (GraphPad Software, Inc., San Diego, CA) for analysis of dose-response curves in the presence of antagonists, a nonlinear analysis based on Schild plot technique. Simultaneous addition of the two peptides may not provide sufficient time for maximum binding of VIP4–28 (Sjoberg et al., 1987).

**Peptide Synthesis.** All peptides were synthesized by automated solid phase techniques in a three-vessel model 430A system (Applied Biosystems) as described previously (Goetzl et al., 1988). After cleavage from the resin with hydrofluoric acid, the peptides were purified by high-performance liquid chromatography (HPLC) on a 2 × 25 cm octadecylsilane column in a model 1406 A system using a solvent program of 30 min of 0.1% trifluoroacetic acid in water at 8 ml/min and then a 90-min gradient of 65% acetonitrile/35% 0.1% trifluoroacetic acid. Identity of each peptide (Fig. 1) was evaluated by complete amino acid sequence with gas-phase Edman method in a model 470A system equipped with on-line narrow bore HPLC analysis (Beckman).
model 120A) and automated data integration (model 900A) for quantification of parathyroid hormone amino acids. All experiments using VIP4–28 were also performed with peptide purchased from Bachem.

Results

VPAC1 Expression on HT-29 Colon Carcinoma Cells and VPAC2 Expression on Molt-4b Lymphoblasts. RNA was harvested from two human cell lines, HT-29 colon carcinoma and Molt-4b lymphoblastic cells, and analyzed by real-time RT-PCR. As shown in Fig. 2, HT-29 cells express VPAC1 (4157 ± 1126 copies/100 pg of rRNA, mean ± S.E., n = 6), but had no detectable VPAC2 mRNA (p < 0.0001). In contrast, Molt-4b cells express VPAC2 mRNA (6099 ± 1440 copies/100 pg of rRNA, mean ± S.E.), but nondetectable levels of VPAC1 (p < 0.0001). Fewer than 50 copies/100 pg of rRNA is considered nondetectable in the real-time PCR conditions used in this protocol. For illustration purposes in Fig. 2 and for statistical analysis, 10 copies/100 pg of rRNA has been arbitrarily designated when copy number is below the limits of detection.

High-Affinity Binding of VIP and VIP4–28 to VPAC1 and VPAC2. Competitive binding studies were performed on plasma membranes harvested from the HT-29 colonic epithelial cell line. VIP4–28 was equally as effective as VIP as a competitive inhibitor of 125I-VIP binding (Fig. 3A). These experiments revealed apparent identity in the affinity of receptors recognized by VIP and VIP4–28 with KD values of 1.6 and 1.7 nM, respectively (Table 1). Using the conversion factor of 1 µg of membrane protein/2.3 × 10^6 HT-29 cells, the Bmax of 0.7 nM extrapolates to 85,000 VPAC1 receptors/cell.

Molt-4b lymphoblasts exhibit a single class of high affinity VIP binding sites with a KD of 1.7 nM for both VIP and VIP4–28 (Fig. 3B). Analysis of the number of binding sites for each peptide using the LIGAND program revealed apparent identity between VIP and VIP4–28 (Bmax = 0.7 ± 0.1 and 0.8 ± 0.6 nM, respectively), suggesting that the two peptides bind to the same receptor (Table 1). Using this method of membrane preparation, 1 µg of membrane protein corresponds to 9.5 ± 2.9 × 10^6 Molt-4b cells. The Bmax of 0.7 nM represents an estimated 20,000 high-affinity VPAC2 binding sites per cell, in good agreement with our previous estimate of 15,000 sites/cell (Beed et al., 1983).

Binding of VIP6–28 and VIP10–28 to VPAC1 and VPAC2. The peptides VIP6–28 and VIP10–28, were less effective inhibitors of 125I-VIP binding in both cell lines (Fig. 3, A and B). VIP6–28 bound to HT-29 colonocytes and to Molt-4b lymphoblasts with intermediate affinities of 7.2 and 8.4 nM, respectively. VIP10–28 demonstrated the lowest affinity for VPAC1 and VPAC2 with KD values of 74 and 67 nM in HT-29 cells and Molt-4b cells, respectively (Table 1). Thus, the three natural variants of VIP demonstrated competitive...
inhibition of 125I-VIP binding with the rank order of potency VIP₄₋₂₈ = VIP > VIP₆₋₂₈ ≫ VIP₁₀₋₂₈.

**VIP₄₋₂₈ Activation of Signal Transduction via VPAC1.** The agonist activities of the VIP variant peptides were compared in cyclic nucleotide experiments (Table 2). The basal cAMP level is higher in the Molt-4b lymphoblastic cell line than in HT-29 colonic cells. However, prostaglandin E₂ induces 16- to 22-fold increases in cAMP in both cell lines and forskolin induces a 150-fold increase in cAMP in both cell lines. In contrast, VIP is a much more effective agonist in HT-29 cells than in Molt-4b cells, raising cAMP levels in HT-29 cells 225-fold over basal in 5 min compared with a 14-fold increase in Molt-4b cells. VIP₄₋₂₈ induces ~400-fold increase in cAMP levels in HT-29 cells, but has no agonist activity in Molt-4b lymphoblasts. VIP₆₋₂₈ has agonist activity similar to VIP in both cell types, whereas VIP₁₀₋₂₈ has no agonist activity in either HT-29 colonic cells or in Molt-4b lymphoblasts.

**VIP₄₋₂₈ Is VPAC1 Agonist and VPAC2 Antagonist.**

Figure 4 demonstrates comparative dose-response curves for VIP₄₋₂₈ in HT-29 colonic cells and Molt-4b lymphoblasts. The basal level of cAMP in HT-29 cells is 0.9 pmol/10⁶ cells at any concentration tested over the range 0.1 nM to 100 nM and 1 μM concentrations (p < 0.01), whereas no significant increase in cAMP levels is observed in Molt-4b cells at any concentration tested over the range 0.1 nM to 1 μM VIP₄₋₂₈.

The dose-response curve for VIP-mediated generation of cAMP via VPAC1 in HT-29 colon carcinoma cells is shown in Fig. 5. VIP is a more efficient agonist than VIP₄₋₂₈. 10 nM VIP induces maximal cAMP accumulation compared with 1 μM VIP₄₋₂₈ (Fig. 5 versus Fig. 4). Also shown in Fig. 5 is the effect of 1 μM VIP₄₋₂₈ together with increasing concentra-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>VIP</th>
<th>VIP₄₋₂₈</th>
<th>VIP₆₋₂₈</th>
<th>VIP₁₀₋₂₈</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT-29 (VPAC1)</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>7.2 ± 4.7</td>
<td>74 ± 71</td>
</tr>
<tr>
<td>Molt-4b (VPAC2)</td>
<td>1.7 ± 0.9</td>
<td>1.7 ± 0.7</td>
<td>8.4 ± 2.5</td>
<td>67 ± 47</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Dose-response effect of VIP₄₋₂₈ on cAMP accumulation in HT-29 colon carcinoma cells and Molt-4b lymphoblasts. HT-29 cells (5 × 10⁵) in monolayers or Molt-4b cells in suspension (1 × 10⁶) were incubated 5 min at 37°C in the presence of the indicated concentration of VIP₄₋₂₈. Cyclic AMP was extracted as described under Materials and Methods and quantified by radioimmunoassay. Results shown are mean ± S.D. of triplicates from one of four experiments, all with similar results. S.D.s for all points in both curves are <2 pmol, except at 100 nM (249 ± 18 pmol) and 1 μM (310 ± 6 pmol) in the HT-29 curve; hence, SDs are encompassed in the respective symbols.

**Fig. 5.** VIP-mediated cAMP accumulation in HT-29 colon carcinoma cells in the presence and absence of VIP₄₋₂₈. HC-29 colon carcinoma cells were incubated at a concentration of 5 × 10⁵ cells/monolayer in media and the indicated concentration of VIP in the presence or absence of 1 μM VIP₄₋₂₈ for 5 min at 37°C. Cyclic AMP was extracted and quantified as described under Materials and Methods. Values are mean ± S.D. for triplicate samples from one of three independent experiments, all with similar results. When not shown, S.D. is included in the symbol.

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**TABLE 1**

Competitive inhibition of 125I-VIP binding by VIP, VIP₄₋₂₈, VIP₆₋₂₈, and VIP₁₀₋₂₈.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Kᵤ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM</td>
</tr>
<tr>
<td>HT-29 (VPAC1)</td>
<td>64 ± 7</td>
</tr>
<tr>
<td>Molt-4b (VPAC2)</td>
<td>5.5 ± 1.2</td>
</tr>
</tbody>
</table>

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**VIP₄₋₂₈ Antagonism of VPAC2 Is Dose-Dependent.**

Dose-response curves for VIP interaction with VPAC2 were generated in Molt-4b cells in the presence of various concentrations of VIP₄₋₂₈. Figure 7 demonstrates VIP dose-response curves in the presence of 0.1 nM, 10 nM, and 1 μM VIP₄₋₂₈. Although VIP was able to induce full agonist activation of VPAC2 in the presence of the putative agonist VIP₄₋₂₈, the slope of the resulting Schild plot is 0.59 ± 0.34.

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*Significantly different from basal level, p < 0.01.
VPAC1 Agonist Activity of VIP<sub>4–28</sub> Is Not Inactivated by Lymphoblasts. One possible explanation for the paradoxical effects of VIP<sub>4–28</sub> in HT-29 cells and Molt-4b cells would be proteolytic degradation of the peptide by lymphoblasts. This possibility was tested in three sets of experiments: 1) addition of protease inhibitors to Molt-4b cells during VIP<sub>4–28</sub> exposure; 2) incubation of VIP<sub>4–28</sub> with Molt-4b cells before addition to HT-29 cells; and 3) extraction and HPLC purification of VIP<sub>4–28</sub> and possible degradation products after incubation with Molt-4b cells.

Addition of protease inhibitors did not enhance the ability of VIP<sub>4–28</sub> to induce cAMP generation in Molt-4b lymphoblasts (Table 3). To further test whether Molt-4b lymphocytes hydrolyze VIP<sub>4–28</sub> to a peptide fragment with antagonist activity in both HT-29 and Molt-4b cells, VIP<sub>4–28</sub> was added to Molt-4b lymphoblast cultures at a concentration of 1 μM. After 5 min, the medium (containing VIP<sub>4–28</sub> and any hydrolyzed peptides) was incubated with HT-29 cells. As can be seen in Table 4, VIP<sub>4–28</sub> had no agonist activity in Molt-4b lymphoblasts, but when transferred to colonic cells, this...
TABLE 3
Effect of protease inhibitors on VIP$_{4-28}$ in Molt-4b lymphoblasts

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>cAMP$_{10^6}$ cells Basal</th>
<th>1 µM VIP</th>
<th>1 µM VIP$_{4-28}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bactracin</td>
<td>2.2 ± 0.2</td>
<td>43.0 ± 1.3</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>PMSF + DFP</td>
<td>2.3 ± 0.1</td>
<td>39.0 ± 6.4</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>PA + β-T</td>
<td>2.5 ± 0.2</td>
<td>12.6 ± 1.4</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Bestatin + pepstatin</td>
<td>1.7 ± 0.4</td>
<td>14.4 ± 1.0</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>PMSF, phenylmethylsulfonyl fluoride; DFP, diisopropyl fluorophosphate; PA, phosphoramidon.</td>
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TABLE 4
Effect of VIP$_{4-28}$ on cAMP levels in colon carcinoma cells and T lymphoblasts

<table>
<thead>
<tr>
<th></th>
<th>cAMP (pmol/10^6 cells) Basal</th>
<th>1 µM VIP</th>
<th>1 µM VIP$_{4-28}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molt-4b</td>
<td>1.9</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>HT-29</td>
<td>11.9</td>
<td>16112.0</td>
<td></td>
</tr>
<tr>
<td>HT-29 + Molt-4b medium</td>
<td>1.9</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

same media (containing VIP$_{4-28}$ and/or its degradation products) demonstrated potent agonist activity in HT-29 cells. Molt-4b cells and HT-29 cells that had been incubated with 1 µM VIP$_{4-28}$ for 5 min at 37°C were extracted in ethanol/acetic acid and the peptides analyzed by HPLC. VIP$_{4-28}$ was the only peptide identified in the extract.

**Discussion**

Endogenous antagonists have now been identified for several receptors, including hepatocyte growth factor (Chan et al., 1991) and the immunoglobulin receptor (Torigoe et al., 1998). These antagonists are pseudoreceptors or truncated receptors. Analogs of a ligand can also function as antagonists; β-blockers that antagonize action of endogenous β-adrenergic receptor ligands are among the most well known antagonists in this category (Strader et al., 1989). Endogenous peptides are physiological antagonists of the IL-1 and IL-7 receptors (Carter et al., 1990; Granowitz et al., 1991).

Several VIP analogs have been designed as VIP receptor antagonists. These peptide ligands include VIP$_{10-28}$ (Turner et al., 1986), (N-Ac-Tyr$^1$,d-Phc$^5$)-GRF(1–29)-NH$_2$ (Waebbroeck et al., 1985), and neurotensin$_{6-11}$-VIP$_{7-28}$ (Gozes et al., 1996). Although VIP demonstrates high-affinity binding with affinity constants in the subnanomolar range, the above-named antagonists all have dissociation constants in the micromolar range. We demonstrate that VIP$_{4-28}$ has affinity equal to VIP, has potent agonist activity at VPAC1, and has potent antagonist activity at VPAC2. The first three amino acids in VIP (H, S, and D), seem to be important for stimulation of adenylate cyclase via VPAC2 as suggested by the ability of both VIP and VIP$_{4-28}$, but not VIP$_{6-28}$ or VIP$_{10-28}$ to induce cAMP accumulation in Molt-4b cells. This is consistent with the early observations of Mutt (1982) that aspartic acid in position 3 at the N-terminal end of VIP and secretin is important in cAMP generation. VIP$_{4-28}$ thus is unique in several respects: 1) it is an endogenous peptide generated by lymphocyte proteolysis of VIP; 2) it binds with high affinity to both VPAC1 and VPAC2; and 3) it has VPAC1 agonist activity and VPAC2 antagonist activity. These observations all suggest a role for VIP$_{4-28}$ in vivo.

Previous studies have established the presence of high-affinity VIP receptors on human intestinal epithelial cells where VIP mediates water and electrolyte secretion as well as on human T and B lymphocytes (Danek et al., 1983; O’Dorisio et al., 1989). In the immune system, VIP seems to modulate lymphocyte trafficking, T cell proliferation, and B cell synthesis of IgA (Lara-Marquez et al., 2001). The present studies used two human cell lines as in vitro models of intestinal epithelial cells and immune cells. The Molt-4b cell line is derived from human leukemia cells. HT-29 is a colonic epithelial cell line established from a human colon carcinoma. Previous studies from our laboratory have demonstrated that VIP interacts with high-affinity receptors to stimulate adenylate cyclase, activate protein kinase A, and induce phosphorylation of an identical 38-kDa protein in both cell lines (O’Dorisio and Campolito, 1989). The results presented here suggest that functional differences exist between the VPAC1 receptor expressed on HT-29 cells and the VPAC2 receptor expressed on Molt-4b lymphoblasts. Although the lymphoblastic and colonic cell receptors seem to bind VIP with equal affinity and with similar numbers of receptors per milligram of membrane protein, the HT-29 receptor complex seems to transduce a signal from VIP and VIP$_{4-28}$ to adenylate cyclase more efficiently.

Although the number of receptors per milligram of membrane protein is quite similar in HT-29 cells and Molt-4b lymphoblasts, the number of VIP receptors per cell is 4-fold greater in HT-29 cells; this may account partially for the higher stimulation index for VIP in HT-29 cells. However, the finding that VIP$_{4-28}$ stimulates cAMP generation in HT-29 cells and inhibits VIP-mediated cAMP accumulation in Molt-4b lymphoblasts demonstrates that VPAC1 and VPAC2 are functionally quite different receptors.

The α and β subtypes of adrenergic receptors were identified by their similar affinities for epinephrine; these subtypes can, however, be differentiated by selective drugs and by their second messenger generation in response to epinephrine. These receptors are now known to be the products of separate but homologous genes. VPAC1 and VPAC2 are products of distinct genes (Adamou et al., 1995). The VPAC1 receptor has been cloned from HT-29 colon carcinoma cells and shown to be a 42-kDa protein (Sreedharan et al., 1991). VPAC2 was cloned from a human placental library (Adamou et al., 1995). Cross-linking studies in our laboratory have demonstrated a 47-kDa receptor protein in both HT-29 and Molt-4b cells (Wood and O’Dorisio, 1985). The results of the present study suggest that VPAC1 on HT-29 colonic epithelial cells and VPAC2 on Molt-4b lymphoblasts can be functionally differentiated by their response to VIP$_{4-28}$.

This may be of functional significance in the intestine wherein VIP modulates water and electrolyte secretion via stimulation of adenylate cyclase in intestinal epithelial cells (Amirnoff et al., 1978). VIP also seems to modulate secretion of IgA, the major antibody in intestinal secretions (Stanisz et al., 1986). In the immune system, VIP synthesized and released from eosinophils modulates cytokine production.
References
Acknowledgments
We acknowledge the advice of Dr. Gerald Gehbart on Schild plot analysis, the expert statistical assistance of John Hayes, and the peptide synthesis by Dr. Christoph W. Turck.
(Weinstock, 1991). VIP down-regulates IL-2, IL-4 (Iwamoto et al., 1992), IL-10 (Martinez et al., 1998), and tumor necrosis factor-α (Dewit et al., 1998; Jabrane-Ferrat et al., 1999) and up-regulates antigen-induced interferon-γ (Jabrane-Ferrat et al., 1999) as well as IL-5 (Mathew et al., 1992). We have shown that VPAC1 is down-regulated and VPAC2 is up-regulated during activation of CD4+ T cells (Lara-Marquez et al., 2001). VIP and/or similar peptides regulate circadian rhythms; mice lacking the VPAC2 gene fail to adapt to changes in light cycles (Harmar et al., 2002).
The observations reported here suggest that VIP released from nerve endings or eosinophils in the gut can differentially activate enterocytes and lymphocytes. In lymphocytes, VIP seems to be hydrolyzed to VIP4-28 by an unknown mechanism (Goetzl et al., 1989). We now demonstrate that the peptide fragment, VIP4-28, stimulates adenylate cyclase in enterocytes via VPAC1 and inhibits VPAC2-mediated stimulation of adenylate cyclase. Thus, the presence of an antagonist for VPAC2 may have functional significance in the intestine wherein VIP modulates water and electrolytes (Barbezat and Grossman, 1971) in enterocytes expressing VPAC1 (Amiranoff et al., 1978) and also comes in contact with intraepithelial lymphocytes expressing VPAC2. VPAC1 and VPAC2 are the only known receptors to which VIP binds with high affinity. Although VIP binds to the pituitary adenylate cyclase activating peptide receptor PAC1, VIP has both lower affinity and lower potency as a PAC1 ligand than does pituitary adenylate cyclase activating peptide (Sano et al., 2002). For this reason, and also because we have no access to a PAC1-expressing human cell line lacking VPAC1 and VPAC2 expression, these studies have not examined the agonist and antagonist activity of VIP, VIP4-28, VIP4-28, and VIP4-28 at the PAC1 binding site. If VIP4-28 proves to be a potent VPAC1 agonist/VPAC2 antagonist in vivo, its agonist/antagonist activity at the PAC1 binding site would be warranted.
In summary, VIP4-28, a proteolytic product of the major secreted peptide VIP1-28, is a potent agonist for VPAC1 and a potent antagonist for VPAC2. This identification of an endogenous VPAC2 antagonist has important implications for selective regulation of intestinal secretion and mucosal function.

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