Expression, pharmacological, and functional evidence for PACAP/VIP receptors in human lung

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1Department of Biochemistry and Molecular Biology, University of Alcalá, 28871 Alcalá de Henares; and 2Department of Morphological Sciences and Surgery, University of Alcalá, 28871 Alcalá de Henares; and 3Department of Thoracic Surgery, Ramón y Cajal Hospital, 28034 Madrid, Spain

Busto, Rebeca, Isabel Carrero, Luis G. Guijarro, Rosa M. Solano, José Zapatero, Fernando Noguerales, and Juan C. Prieto. Expression, pharmacological, and functional evidence for PACAP/VIP receptors in human lung. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L42–L48, 1999.—Pituitary adenylate cyclase-activating peptide (PACAP) type 1 (PAC 1) and common PACAP/vasoactive intestinal peptide (VIP) type 1 and 2 (VPAC 1 and VPAC 2, respectively) receptors were detected in the human lung by RT-PCR. The proteins were identified by immunoblotting at 72, 67, and 68 kDa, respectively. One class of PACAP receptors was defined from 125I-labeled PACAP-27 binding experiments (dissociation constant = 5.2 nM; maximum binding capacity = 5.2 pmol/mg protein) with a specificity: PACAP-27 ≈ VIP > helodermin > peptide histidine-methionine (PHM) ≫ secretin. Two classes of VIP receptors were established with 125I-VIP (dissociation constants of 5.4 and 197 nM) with a specificity: VIP = helodermin = PACAP-27 ≫ PHM ≫ secretin. PACAP-27 and VIP were equipotent on adenylyl cyclase stimulation (EC50 = 1.6 nM), whereas other peptides showed lower potency (helodermin > PHM > secretin). PACAP/VIP antagonists supported that PACAP-27 acts in the human lung through either specific receptors or common PACAP/VIP receptors. The present results are the first demonstration of the presence of PAC 1 receptors and extend our knowledge of common PACAP/VIP receptors in the human lung.

Pituitary adenylate cyclase-activating peptide; vasoactive intestinal peptide; signal transduction; pituitary adenylate cyclase-activating peptide type 1 receptor; adenylyl cyclase

VASOACTIVE INTESTINAL PEPTIDE (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) are widely distributed neuropeptides belonging to the same family of peptides as secretin and glucagon (1). VIP is a 28-amino acid peptide with a 68% sequence homology with PACAP-27 and a widespread distribution in the peripheral nervous system. VIPergic fibers are present in the upper airways and tracheobronchial tree and are particularly abundant around seromucous glands and smooth muscle (15). VIP appears to be the major peptide transmitter of the nonadrenergic noncholinergic inhibitory component of autonomic innervation of the lung where it acts as a potent smooth muscle relaxant and induces bronchodilation and vasodilation (27). VIP also exerts an antiproliferative effect on human airway smooth muscle cells (16). Because airway smooth muscle proliferation is a major feature of bronchial asthma, the observed VIP deficiency in the airways of severe asthmatic patients could explain the hyperresponsiveness and hyperplasia of airway smooth muscle cells in that disease (22). Interestingly, VIP has recently been shown to suppress the growth and proliferation of human small cell lung cancer (SCLC) as studied on tumor cell implants in athymic nude mice (17). On the other hand, nerve fibers displaying PACAP immunoreactivity have been found in the respiratory tract of different species (rat, guinea pig, ferret, pig, sheep, and squirrel monkey) (36). In humans, PACAP-containing nerve fibers are abundant in the tracheal and bronchial walls, located among smooth muscle bundles and around glands and small blood vessels (15). PACAP has been described as a potent relaxant of tracheal smooth muscle, and it also possesses anti-inflammatory activity (3).

The cDNAs for three distinct PACAP/VIP receptors have been cloned (10): the PACAP type 1 (PAC 1) receptor (which stimulates both adenyl cyclase and phospholipase C) has a higher affinity for PACAP (27 or 38) than for VIP, whereas the common PACAP/VIP type 1 and 2 (VPAC 1 and VPAC 2, respectively) receptors have equal affinity for these two peptides (4, 34). VPAC 2 probably corresponds to the “helodermin-prefering” VIP binding site. Specific receptors for VIP have been identified in membrane preparations from rat and human lungs (23, 24) as well as in non-small cell lung cancer (NSCLC) and SCLC cell lines (13, 29). PACAP binds to a lung receptor that until now is believed to be a VIP receptor that recognizes with nearly equal affinity VIP, PACAP-27, and PACAP-38. PACAP receptors have been described in the rat lung (11) and in human SCLC and NSCLC cell lines (19, 38) where they can regulate tumor cell growth and differentiation.

Despite the existence of PACAP-containing fibers innervating human airways, there are no reports at present on the existence of specific PACAP receptors in the human lung and the functional role of PACAP in the respiratory system. The close structural and functional relationship between PACAP and VIP and the coexistence of both peptides in lung nerve fibers (15) increase the interest in these peptides because of the possibility of their concomitant action at this level. In the present report, we studied the expression and characteristics of PACAP receptors and the corresponding signal transduction step involved in the action of this peptide in the human lung. For comparative purposes, we also ran experiments in parallel on rat lung membranes.
MATERIALS AND METHODS

Reagents. VIP, peptide histidine-methionine (PHM), and secretin were purchased from Neosystem (Strasbourg, France). PACAP-27 and anti-rabbit IgG, heavy and light chain (goat) peroxidase conjugated, were from Calbiochem-Novabiochem (San Diego, CA). PACAP-(6–38) was from American Peptide (Sunnyvale, CA). Helodermin was from Peninsula (St. Helens, UK). [Lys1,Pro2,5,Arg3,4,Tyr6]VIP, creatine phosphate, phosphocreatine kinase, IBMX, Triton X-100, and chemicals for SDS-PAGE were from Sigma (Alcobendas, Spain). Protein markers for SDS-PAGE were from Bio-Rad (Hercules, CA). The specific antisera for VIP and PACAP receptors were generous gifts from Dr. E. J. Goetzl (University of California, San Francisco, CA) and Dr. A. Arimura (Tulane University, Belle Chasse, LA), respectively. The peroxidase system Supersignal substrate for Western blotting was from Pierce (Rockford, IL). The Ultraspec kit for RNA extraction was from Biotec (Houston, TX). The first-strand cDNA synthesis kit for RT-PCR [avian myeloblastosis virus (AMV)] was from Boehringer Mannheim (Barcelona, Spain). Oligonucleotides for PCR were synthesized by Pharmacia (Uppsala, Sweden). 5′- and 3′-mercaptotethanol, and 0.05% bromphenol blue, and the test substances (VIP, PACAP-27, helodermin, or secretin). At the end of incubation, the samples were filtered under vacuum with Whatman GF/C filters for nonspecific binding. Nonspecific binding (determined in the presence of 1 µM VIP) was subtracted from the total binding to obtain the specific binding. 125I-VIP and 125I-PACAP-27 were prepared from male Wistar rats weighing 300–350 g.

PACAP/VIP binding studies. Human lung (0.04–0.06 mg protein/ml) or rat lung (0.04–0.08 mg protein/ml) membranes were incubated for 45 min at 15°C with 150 pM 125I-VIP in 0.25 ml of 25 mM Tris-HCl buffer (pH 7.4) containing 5 mM MgCl2, 0.1% bacitracin, 0.5% BSA, and increasing concentrations of PACAP-27, VIP, PHM, or secretin. At the end of incubation, the samples were filtered under vacuum with Whatman GF/C filters pretreated with 0.5% polyethyleneimine. Washed twice with 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 mM EDTA and 0.2% BSA, and counted for radioactivity. Nonspecific binding (determined in the presence of 1 µM VIP or PACAP-27) averaged ~4% of the total radioactivity in both cases and was subtracted from the total binding to obtain the specific binding. 125I-VIP and 125I-PACAP-27 were prepared with the chloramine T method at a specific activity of ~250 Ci/g (31).

Adenylyl cyclase assay. As previously described (22), human or rat lung membranes (0.01–0.02 mg protein/ml) were incubated in 25 mM triethanolamine-HCl buffer (pH 7.4) containing 1.5 mM ATP, an ATP-regenerating system (7.4 mM creatine phosphate and 1 mg/ml of creatine kinase), 5 mM MgSO4, 1 mM IBMX, 1 mM EDTA, 1 mg/ml of bacitracin, and the test substances (VIP, PACAP-27, helodermin, PHM, secretin, or forskolin) in 0.1-ml final volume. After 30 min of incubation at 30°C, the reaction was stopped by heating the mixture for 3 min at 100°C. After the addition of 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine-HCl buffer, pH 7.4) and centrifugation at 3,300 g for 10 min at 4°C, the supernatant was taken for the assay of cAMP.

Immunodetection of PACAP/VIP receptors. Human lung membranes were solubilized in 50 mM Tris-HCl buffer (pH 7.4), 1% (vol/vol) Triton X-100, and 0.01% trypsin inhibitor for 30 min at 4°C. After centrifugation at 38,000 g for 15 min at 4°C, the supernatant was mixed with the same volume of 50 mM Tris-HCl buffer containing 1% (vol/vol) Triton X-100, and 0.01% trypsin inhibitor for 30 min at 4°C. After centrifugation at 38,000 g for 15 min at 4°C, the supernatant was mixed with the same volume of 50 mM Tris-HCl buffer containing 20% glycerol, 6% SDS, 10% β-mercaptoethanol, and 0.05% bromphenol blue, and the proteins were run on 10% SDS-PAGE. After transfer of the proteins to nitrocellulose sheets, PACAP/VIP receptors were immunodetected with rabbit anti-human PAC1 (30), VPAC1, and VPAC2 (7) receptor sera. To allow for the recognition of nonspecific staining, the primary antibodies were also used after preabsorption with the corresponding synthetic peptides used for immunization. The immunoreactive proteins were revealed with peroxidase-conjugated goat anti-rabbit IgG and analyzed by luminescence according to a standard protocol from Pierce.

RESULTS

Identification of PACAP/VIP receptor mRNAs. We used the RT-PCR methodology to determine the expression of the different PACAP/VIP receptors in the human lung. With the appropriate cDNA (i.e., cDNA giving a positive signal for β-actin amplification) from four different samples studied, RT-PCR identified PAC1+, VPAC1+, and VPAC2+ receptor mRNAs. RT-PCR products had the expected sizes, and two bands were detected for PAC1 receptor in three samples, corresponding to the normal (null) PAC1 receptor (304 bp) and the SV-1 or SV-2 splicing variants (386 bp) (Fig. 1).

Immunodetection of PAC1+, VPAC1+, and VPAC2+ receptors. A protein of ~72-kDa molecular mass was detected by means of a rabbit antiserum against a synthetic peptide corresponding to positions 411–453 (COOH-terminal intracellular domain) of the PAC1 receptor (30). However, the use of nonreducing conditions showed proteins with greater electrophoretic mobility (molecular mass of 58 kDa; data not shown), supporting the presence of intramolecular disulfide bridges in the PAC1-receptor molecule.

Immunodetection of the human PACAP/VIP receptors VPAC1 and VPAC2 was carried out by using the
anti-human VPAC1-receptor peptide A (which recognizes the first extracellular loop, amino acids 191–222) and B (which binds to the carboxy-terminal cytoplasmic tail, positions 391–457) antibodies and VPAC2-receptor peptide 4 (which recognizes the amino terminus, positions 54–70), 6 (against the first extracellular loop, amino acids 191–222), and 22 and 23 (which bind to the extracellular amino terminus, positions 19–37 and 67–83, respectively) antibodies (7). Peptide A and B antibodies detected a protein of ~67 kDa, whereas peptide 4, 6, 22, and 23 antibodies detected proteins of 68 kDa. The specificity of the antibodies was established by their preincubation with the corresponding immunizing peptides: the preabsorption step completely abolished the immunostaining. Figure 2 shows the results from representative experiments with the anti-human PAC1-receptor peptide, VPAC1-receptor peptide B, and VPAC2-receptor peptide 22 antibodies.

Receptor binding and specificity of peptide binding. We studied the specific binding of 125I-PACAP-27 and 125I-VIP as well as the pharmacological profile of binding for different structurally related peptides. 125I-PACAP binding was competitively inhibited by unlabelled PACAP-27 and VIP, which were equipotent, whereas helodermin and PHM exhibited a 15 times lower potency and secretin displaced 125I-PACAP with a very low affinity (it did not achieve the 50% of displacement at the highest concentration used; Fig. 3, Table 1). Scatchard analysis of the data gave a straight line, indicating that PACAP binds to a single population of sites. The data were analyzed with the least-squares nonlinear regression computer program LIGAND (20). From five distinct human lung samples, the dissociation constant (K_d) was estimated at 5.2 ± 1.4 nM and the maximum binding capacity (B_max) at 5.2 ± 1.1 pmol/mg membrane protein.

Similar studies were carried out in the rat lung where the potency of the different peptides to inhibit 125I-PACAP binding gave the results shown in Fig. 3 and Table 1: PACAP = VIP = PHM = secretin. In the rat, Scatchard analysis of PACAP binding gave a curvilinear plot that was interpreted by the LIGAND program in terms of two classes of PACAP receptors possessing different affinities: a first site with high affinity (K_d = 0.65 ± 0.13 nM) and a low-binding capacity (6.3 ± 1.3 pmol/mg protein) and a second site with low affinity (K_d = 185 ± 15.3 nM) and a high-binding capacity (83.2 ± 7.4 pmol/mg protein).

Stoichiometric experiments were also performed on human and rat lung membranes with a fixed concentration of 125I-VIP and increasing doses of unlabeled ligand (Fig. 4). The corresponding Scatchard analysis for VIP binding gave upwardly concave curves. The data in human samples (n = 4) were interpreted as above and resolved into high-affinity (K_d = 5.4 ± 3.3 nM; B_max = 7.1 ± 2.8 pmol/mg protein) and low-affinity (K_d = 197 ± 30.8 nM; B_max = 138 ± 15.5 pmol/mg protein) binding sites. Rat preparations also resolved into high-affinity (K_d = 0.44 ± 0.11 nM; B_max = 5.3 ± 1.9 pmol/mg protein) and low-affinity (K_d = 124 ± 21.3 nM; B_max = 110 ± 11.5 pmol/mg protein) binding sites. The pharmacological features of 125I-VIP binding to human and rat lung membranes were analyzed in competitive experiments with various VIP structurally related peptides (Fig. 4). As shown by the mean IC_50 values (Table 2), there were similar patterns in the specificity of these ligands for VIP receptors because, in both the human and rat, the order of potency of the different peptides was VIP = helodermin ≈ PACAP-27 ≈ PHM ≈ secretin.

Fig. 1. RT-PCR analysis of pituitary adenylate cyclase-activating peptide (PACAP) type 1 (PAC1; lane 1) and common PACAP/vasoactive intestinal peptide (VIP) type 1 and 2 (VPAC1; lane 2) and VPAC2 (lane 3) respectively receptor mRNAs in human lung. cDNA generated from total RNA was amplified with specific primers. Nos. at left, DNA standard molecular-mass markers (lane M) shown as base pairs. Data correspond to a representative experiment of 4 patients tested.

Fig. 2. Immunoblot detection of PAC1, VPAC1, and VPAC2 receptors in human lung membranes. Lanes 2, membranes (50 µg of protein) were resolved and immunoblotted with antisera to PAC1, VPAC1 (peptide B), and VPAC2 (peptide 22) receptors as described in MATERIALS AND METHODS. Lanes 1, specificity controls of preincubation of each antibody with corresponding immunizing peptide. No. at left, reference protein-size marker. Experiments are representative of 5 performed with different patients.
secretin but with a higher affinity in the rat than in the human samples.

Functionality of PACAP/VIP receptors in adenylyl cyclase stimulation. The signaling pathway of the different peptides studied is functionally coupled to the adenylyl cyclase system as shown in experiments with increasing doses of these agonists (Fig. 5). VIP and PACAP had the same efficacy and potency (EC50 1.65 nM), whereas other related peptides were clearly less potent and/or efficacious in enzyme stimulation: helodermin EC50 5 14.7 nM, PHM EC50 5 100 nM, and secretin EC50 5 775 nM. Maximal stimulation of adenylyl cyclase activity was achieved with forskolin, whereas the PACAP/VIP highest level of activity was ~50% of this maximum. No additivity was found between VIP and PACAP on the stimulation of adenylyl cyclase (data not shown).

Effects of VIP and PACAP antagonists on VIP- and PACAP-induced adenylyl cyclase stimulation. To clarify whether PACAP and VIP have an independent pathway in the stimulation of this enzyme, we tested the effects of the VIP and PACAP antagonists [Lys1,Pro2,5,Arg3,4,Tyr6]VIP (9) and PACAP-(6—38) (26), which by themselves had no effect on cAMP (data not shown), on the PACAP- and VIP-induced adenylyl cyclase activity. As shown in Fig. 6, when VIP stimulated adenylyl cyclase, both antagonists produced a 25% decrease in the enzyme activity. However, when PACAP was used to activate this system, PACAP-(6—38) exerted a clear inhibition, whereas the VIP antagonist did not have any effect on PACAP stimulation.

**DISCUSSION**

VIP exerts important biological effects on lung structures, including relaxation of airways and vascular smooth muscle, regulation of microvascular tone and permeability, regulation of mucus secretion, and inhibition of the release of macromolecules from mucus-secreting glands (16, 27). A VIP deficiency in the airways of severe asthmatic patients has been reported (22). Moreover, recent data show inhibitory activity of VIP on SCLC proliferation, indicating the potential usefulness of this peptide as an antineoplastic agent, at

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC50, nM</th>
<th>Human Lung</th>
<th>Rat Lung</th>
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<tbody>
<tr>
<td>PACAP-27</td>
<td>3.2</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>4.5</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Helodermin</td>
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<td>12.6</td>
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<td>PHM</td>
<td>68.0</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Secretin</td>
<td>794</td>
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</table>

Values are means. PACAP, pituitary adenylate cyclase-activating peptide; VIP, vasoactive intestinal peptide; PHM, peptide histidine-methionine. Competition experiments with 125I-PACAP and structurally related peptides represented in Fig. 3 were used to calculate the corresponding IC50 values.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC50, nM</th>
<th>Human Lung</th>
<th>Rat Lung</th>
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<tbody>
<tr>
<td>VIP</td>
<td>5.4</td>
<td>0.4</td>
<td></td>
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<tr>
<td>PACAP-27</td>
<td>10.0</td>
<td>0.6</td>
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<tr>
<td>Helodermin</td>
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<tr>
<td>PHM</td>
<td>123</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Secretin</td>
<td>1,521</td>
<td>35.5</td>
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</table>

Values are means. Competition experiments with 125I-VIP and structurally related unlabeled peptides shown in Fig. 4 were used to calculate the reported IC50 values.
least in some forms of lung cancers (17). However, the role of the new members of the VIP family remains to be established at this level. The existence of a rich supply of PACAP-immunoreactive fibers in human airways (15) gives a morphological basis for PACAP-mediated control of the physiology of human lung, but in contrast with VIP, only sparse data are available on PACAP in the human lung.

RT-PCR experiments showed the mRNA expression of the three classes of PACAP/VIP receptors (PAC1, VPAC1, and VPAC2) in the human lung. Previously, Northern blot analysis revealed the expression of a VPAC1 transcript (32), and VPAC2 has also been detected by the RNase protection assay (37) in this tissue. We used normal, cancer-free lung tissue that was carefully dissected out from pieces obtained during segmentectomy or lobectomy in human patients with bronchopulmonary cancer. However, we cannot absolutely discard the possibility that PACAP receptors could be induced in lung cancer patients and may not be present in the healthy human lung. Studies by our laboratory are in progress to show the distribution of these peptide receptors in different human lung cancers as well as in pieces of normal lung obtained from organs that did not present this pathology.

The human PACAP receptor, similar to the rat PACAP receptor, can be expressed as different splice variants as a result of the variable expression of two cassettes of 28 amino acids within the third cytoplasmic loop encoded by two exons that are alternatively spliced (25). The position of PAC1 primers used in our experiments includes the site of insertion of the SV-1 and/or SV-2 exons and may detect the different splice variants of the PAC1 receptor. Hence, two splice variants were obtained for PAC1 (null and SV-1 and/or SV-2), in agreement with previous observations on the human retina (21).

In immunodetection experiments with a specific antibody, we identified the PAC1 receptor in human lung as a protein of ~72-kDa molecular mass, which is comparable to the molecular mass reported from other human tissues (2). Immunodetection of VPAC1 and VPAC2 gave the molecular masses previously described in a cross-linking study (5).

The study of the pharmacological profile of the specific PACAP receptor (PAC1) by 125I-PACAP-27 binding to membranes from human lungs showed that PACAP-27 and VIP were nearly equipotent. It has been established that PAC1 receptors present a greater affinity for PACAP than for VIP, which does not agree with present pharmacological data on the displacement of 125I-PACAP-27. On the other hand, the PACAP receptor appears to be a single class of high-affinity binding sites, as indicated by Scatchard analysis, although the possibility of the existence of low-affinity binding sites cannot be discarded. The high-affinity PACAP-specific binding site represents only a small fraction (3.5%) of the total PACAP/VIP binding sites, but in addition to the variable action of PACAP via PAC1-receptor splice variants, PACAP has the further potential for inducing a variety of responses through its ability to interact with the VPAC1 and VPAC2 receptors.

The VIP receptor has been previously characterized in the human lung (5, 28) as a functional protein coupled to the adenyl cyclase system (35). The present study defines a high-affinity binding site with a Kd of 5.4 nM and a density of 7.1 pmol/mg protein. These data and the observed IC50 values for VIP, PACAP, and secretin agree with previous observations in the NSCLC NCI-H592 cell line (18).

The experiments performed for comparative purposes in rat lung membranes indicated that the Kd of the high-affinity sites was 0.44 nM for VIP and 0.65 nM for PACAP, whereas the number of receptors was not far from that seen for the corresponding human sites. The pharmacological profile of rat versus human receptors was similar for VIP but showed different affinities for helodermin and PHM in displacing 125I-PACAP-27. In the rat, we found two classes of binding sites (with high and low affinity) for VIP and PACAP as interpreted by Scatchard analysis.
The results obtained in the experiments with PACAP/VIP antagonists agree with those showing a higher affinity of PACAP-(6–38) for PAR1 and PAR2 (IC50 = 40 nM) than for VPAC1 (IC50 = 600 nM) receptors (8). They are also compatible with previous data on osteoblast-like cells with the same antagonists (33) because the competitive antagonist PACAP-(6–38) markedly inhibited PACAP-induced adenylyl cyclase activity but exerted little effect on VIP-induced enzyme stimulation, whereas the VIP antagonist caused a low level of inhibition when VIP stimulated adenylyl cyclase and it practically did not modify the PACAP effect at this level. These findings strongly support that PACAP-27 stimulates adenylyl cyclase via specific receptors as well as via the common VIP receptors. An apparent discrepancy was seen when the binding profiles of the various peptides tested were compared with the corresponding activity profiles on adenylyl cyclase stimulation in human lung membranes: helodermin was about 50% as active as PACAP-27, but it was less potent than these two peptides on the displacement of 125I-PACAP-27 binding. In fact, these observations may be interpreted as other evidence of the presence and functionality of the VPAC2 receptor in the human lung. The VPAC2 receptor corresponds to the helodermin-prefering receptor previously characterized on the basis of the relative potency of natural and synthetic VIP analogs in various systems including lung cancer-derived cell lines (14). Functional coupling to the adenylyl cyclase system also showed a lack of additivity for VIP and PACAP action, but we cannot discard the possibility of the coupling of the PACAP receptor to another transduction system (i.e., phospholipase C) or the involvement of a limiting adenyl cyclase activity. It is known that PACAP can regulate tumor cell growth and differentiation through c-fos-mediated pathways, and PACAP-(6–38) has been shown to inhibit a PACAP-induced increase in c-fos mRNA (6).

The results reported in this study show the complexity of PACAP/VIP receptors in the human lung. It is necessary to discern whether some effects of VIP in the human lung could be accomplished by PACAP through specific receptors because the development of more potent PACAP/VIP-related analogs or peptide mimetics will permit a potential antiasthma or antitumor strategy, with more selective compounds having therapeutic possibilities.

R. Busto and J. Carrero contributed equally to this study.

We are greatly indebted to Dr. A. Arimura (Tulane University, Belle Chasse, LA) and Dr. E. J. Goetzl (University of California, San Francisco, CA) for generously supplying the specific peptides against the human pituitary adenylyl cyclase-activating peptide/vasoactive intestinal peptide receptors.

This work was supported by Dirección General de Investigación Científica y Técnica (Grant PB94-0360), Universidad de Alcalá (Grant E03797), and Consejería de Educación y Cultura de la Comunidad de Madrid (predoctoral fellowship to R. Busto).

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Received 9 December 1998; accepted in final form 11 March 1999.

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