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Antagonistic Effect of a Vasoactive Intestinal Peptide Fragment, Vasoactive Intestinal Peptide(1–11), on Guinea Pig Trachea Smooth Muscle Relaxation

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SUMMARY

The conformation of various regions of vasoactive intestinal peptide (VIP) has been analyzed by semiempirical methods, CD, and NMR spectroscopy, indicating that residues 11–21 are most likely to be helical, whereas the amino-terminal portion VIP(1–11) could exhibit two β-turn structures. VIP(1–11) inhibits ^{125}I-VIP binding to intact guinea pig tracheal epithelial cells and the VIP-induced smooth muscle response. However, the endecapeptide exhibits no effect on the muscle tone. All these data suggest that VIP(1–11) may be a useful tool in studying VIP receptor recognition, its regulation, and cellular functions.

VIP is a 28-amino acid peptide (HSDAVFTDNYTRLRKQMAVKKYLNSILN-amide) first isolated from porcine duodenum by Said and Mutt (1). Neurons containing VIP-like immunoreactivity are widely distributed in both central and peripheral nervous systems (2). VIP is known to influence numerous biological functions. Among them, the regulation of tracheobronchial tone by VIP has been particularly well studied on smooth muscle layers (3), submucosal glands (4), and arterial walls (5).

Because it is an obligatory step for its activity, the binding of VIP to specific receptor sites represents a significant event whose characterization is an elegant means to define more clearly the mechanism by which VIP displays its physiological properties. Regarding this aspect, it is noteworthy that only a few investigations have been devoted to the identification of specific antagonistic or agonistic compounds whose use would allow the clarification of the molecular events leading to the activation of signal-transducing pathways.

The molecular characteristics of VIP receptors, structural requirements for receptor occupancy, and second messenger activation have usually been studied through the inhibition of ^{125}I-VIP binding or stimulation of adenylate cyclase by VIP-related peptides such as glucagon, secretin, or peptide histidine isoleucine. This has led to the discovery of the importance of the four-amino acid fragment of the Nt part of VIP (His-Ser-Asp-Ala). Previous studies (6) on the conformational structure of peptide T, which is responsible for CD4 receptor recognition, gave support to the VIP(1–11) study. Indeed, the pentapeptide sequence of human immunodeficiency virus gp120 (T^4-T^5-N^6-Y^7-T^8) is homologous to residues 7–11 of VIP (T^7-D^8-N^9-Y^{10}-T^{11}). It has been shown that all five amino acid residues contributed to a semirigid conformation of the molecule, so that the sequence was able to recognize the receptor site, involving hydroxyl groups exposed at the periphery of the pentapeptide. Thus, on the basis of these results and conformational calculations, there is evidence that residues 1–4 and 7–11 of VIP adopt a nonrandom structure. Interestingly enough, this sequence could be released by proteases present in the respiratory tract, and the subsequent antagonistic effect might, in some malignancies, add to VIP deficiency. In this paper, the synthesis and the conformational study of VIP(1–11), its binding to tracheal epithelial cell receptors, and its functional characteristics on tracheal smooth muscle (i.e., the regulatory effect of VIP) are reported. It is known that, in tissues, the secretion of hormones by epithelial cells may be involved in the relaxation phenomenon (7). Many binding studies have been performed with different epithelial cell lines, such as intestinal and cervical epithelia (8, 9), in which VIP receptors have been characterized. One of the original contributions of this work is to demonstrate that, in tracheal tissue, epithelial cells are an important target for VIP activity.

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ABBREVIATIONS: VIP, vasoactive intestinal peptide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMSO, dimethylsulfoxide; BSA, bovine serum albumin; MEM, minimum essential medium; COSY, correlation spectroscopy; Nt, amino-terminal; Ct, carboxy-terminal; Fmoc, *N*-(9-fluorenylmethyloxycarbonyl).

Experimental Procedures

Materials

Male guinea pigs (300–350 g) were from Charles River (Saint Aubin les Elbeu, France). [D-*p*-Cl-Phe[6],Leu[17]]-VIP, HEPES, collagen, BSA (fraction V), hydrocortisone, insulin, and purified Pronase were from Sigma (St. Louis, MO), purified collagenase was purchased from Boehringer (Mannheim, Germany), and Dulbecco's MEM and other cell culture reagents were from GIBCO (Grand Island, NY). DMSO-d_6 was from the Commissariat à l'Energie Atomique (Gif sur Yvette, France). Human [3-*iodotyrosyl*-^{125}I]VIP was from Amersham Corporation (Buckinghamshire, UK); human VIP and VIP(10–28) were obtained from Interchim (Montluçon, France). VIP(1–11) was synthesized by the method of Merrifield (10), with Fmoc amino acids obtained from Novabiochem; the synthesis was carried out on a Biolynx 4175 peptide synthesizer coupled with an UV Gilson holochrome HD detector. To assess airway reactivity, tracheal strips were suspended in Krebs Henseleit buffer (pH 7.4) containing 120.8 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 24.9 mM $NaHCO_3$, 2.4 mM $CaCl_2 \cdot 2H_2O$, and 5.6 mM glucose. The enzymatic study was performed with human leukocyte cathepsin G, a gift from Dr. M. Davril (INSERM U16, Lille, France). All other reagents were of the highest available grade and purchased from standard sources.

Methods

Conformational analysis. Among the different algorithms allowing a conformational prediction based on amino acid sequence, the Chou and Fasman procedure (11), the Novotny and Auffray method (12), and the hydrophobic cluster analysis method, described by Gaboriaud *et al.* (13), were chosen.

Spectroscopic studies were performed by using CD and NMR. The CD spectra were recorded with a RJ Mark III Jobin-Yvon instrument. A 0.01-cm cell and concentrations of 1.3×10^{-4} M were used for the measurements. VIP(1–11) was studied in a 10% DMSO/H_2O mixture, and molar ellipticities were reported in degrees $cm^2 \times dmol^{-1}$.

For NMR study, the 11-residue segment was dissolved in DMSO-d_6 (5 mg/ml) under argon atmosphere. ^{1}H chemical shifts were measured in δ ppm, and the DMSO-d_6 residual signal was taken as reference at 2.5 ppm. All spectra were recorded with a Brucker AM 400 WB spectrometer equipped with an Aspect 3000 computer, operating in the Fourier transform mode with quadrature detection at 400 MHz for protons. Connectivities between the amide protons and their Hα protons were established by two-dimensional COSY, using the pulse sequence D1-90°-D2-45° acquisition.

Airway epithelial cell isolation and culture conditions. Epithelial cells were isolated using a modified Wu procedure (14). The excised guinea pig trachea was cannulated by its laryngeal end, rinsed with cold calcium-free MEM, filled with 0.1% protease in MEM, and tightened at the distal end. After a 16-hr digestion at 4°, cells were flushed with cold MEM containing 10% fetal calf serum and centrifuged at 150 × *g* for 10 min at 4°, and the cell pellet was resuspended in Dulbecco's MEM with 10% fetal calf serum, 5 μg/ml insulin, and 1 μM hydrocortisone. The dissociated cells were then plated on tissue culture dishes, which were first coated with collagen (0.01%, 6 μg/cm^2), and incubated at 37° until confluence (48 hr).

Binding studies. Cells (100–120 μg of protein/ml) were washed with a Hanks' solution buffered with 20 mM HEPES (pH 7.4) and were then incubated in the same buffer containing 1% BSA (15) with increasing concentrations of ^{125}I-VIP (1–300 pM), in the absence or presence of unlabeled VIP (1 μM). Competitive binding studies were performed with ^{125}I-VIP (100 pM) and varying concentrations of VIP(1–11) (0–100 μM). Incubations were carried out at 37° for 60 min, in a total volume of 500 μl. Reactions were stopped by addition of ice-cold HEPES-BSA buffer (500 μl), the cells were then washed with 2 × 500 μl of buffer and lysed with deoxycholate (10 mg/ml) and versene-trypsin (0.1%/0.25%), and radioactivity was determined in a γ counter.

Tracheal preparations. Guinea pigs (250–350 g) were killed by cervical dislocation and tracheas were dissected out and immersed in Krebs buffer. They were cleaned of connective tissues and opened longitudinally through the cartilage, and the strip preparation protocol described by Tschichart *et al.* (16) was then carried out. Briefly, five strips corresponding to four cartilaginous rings were cut, suspended in organ baths containing buffer solution, at 37°, and gassed with a mixture of 95% air and 5% CO_2. Tissues were washed three times, and a 2-g tension was progressively applied to the tracheal preparations. To test the reactivity of strips without epithelium, the luminal surface was rubbed gently with a cotton swab. Responses to cumulative additions of carbachol (positive standard of reactivity), VIP, and VIP(1–11) have allowed the plotting of concentration-response curves.

Results

Conformational analysis. The theoretical conformation of the VIP molecule in its entirety was first studied, in order to understand the molecular recognition between the peptide and its receptor. This molecular conformation approach used three different algorithms to determine the possible secondary structure of VIP. First, the Chou and Fasman method (11) led us to predict two β-turns in the Nt segment of the molecule [VIP(1–4), $\langle P_t \rangle = 2.02 \times 10^{-4}$; VIP(7–10), $\langle P_t \rangle = 2.26 \times 10^{-4}$] and a helical organization of the Ct moiety [VIP(12–23), $\langle p_\alpha \rangle = 1.13$, $\langle p_\beta \rangle = 1.02$, $\langle p_t \rangle = 0.79$]. Second, the Novotny and Auffray method (12) showed the propensity of the residue 6–9 sequence to adopt a β-turn conformation, with residues 11–21 being helical. This algorithm is of interest also for the charged residues and hydrophobicity profiles. The Nt segment 1–10 has two negative regions separated by hydrophobic residues; amino acids 11–21 were alternating hydrophilic and hydrophobic residues, whereas the Ct part was of rather hydrophobic nature. These data are in agreement with previous studies, in which a potential amphiphilic π helix starting from the 12th residue has been observed (17). By the third method, hydrophobic cluster analysis (13), based on two-dimensional representation of the sequence, VIP is predicted to be helical in the residue 5–27 segment.

The three computation procedures emphasize the concept of a well ordered molecule consisting of two different parts, the Nt portion, which probably adopts a β-turn structure, and the Ct moiety, which is mainly helical.

The Nt part of VIP appears to play an important role in the molecular recognition of the receptor, so that we focused our study on the residue 1–11 fragment. First, CD and NMR experiments were conducted on VIP(1–11) and related fragments, in order to confirm the conformational predictions.

CD experiments were done in 10% aqueous DMSO on two different fragments, VIP(1–11) and VIP(7–11). In the far-UV region, each spectrum suggests an ordered structure, but without α-helix or β-strand evidence. More precisely, VIP(7–11) must be presumed to be a β-turn structure characterized by a positive dichroic band ($[\theta] = +16{,}300$ deg·cm^2/dmol) located at 217 nm (Fig. 1). In the case of VIP(1–11), the spectrum obtained reflects the presence of several structures possibly including β-turns, because some well characterized bend models (types I, II′, and III) have been reported as giving "α-helix-like" CD spectra. On the other hand, a small negative dichroism in the 250–300-nm region has been observed for VIP(1–11) but not for VIP(7–11) (data not shown). This means that Tyr[10] lies in a specific environment in VIP(1–11) (presumably close to His[1]), thus implying the chain reversal and corroborating results previously obtained by Fournier *et al.* (18).

Simultaneously, the structure of VIP(1–11) was studied using NMR spectroscopy. The presence of intramolecularly hydro-

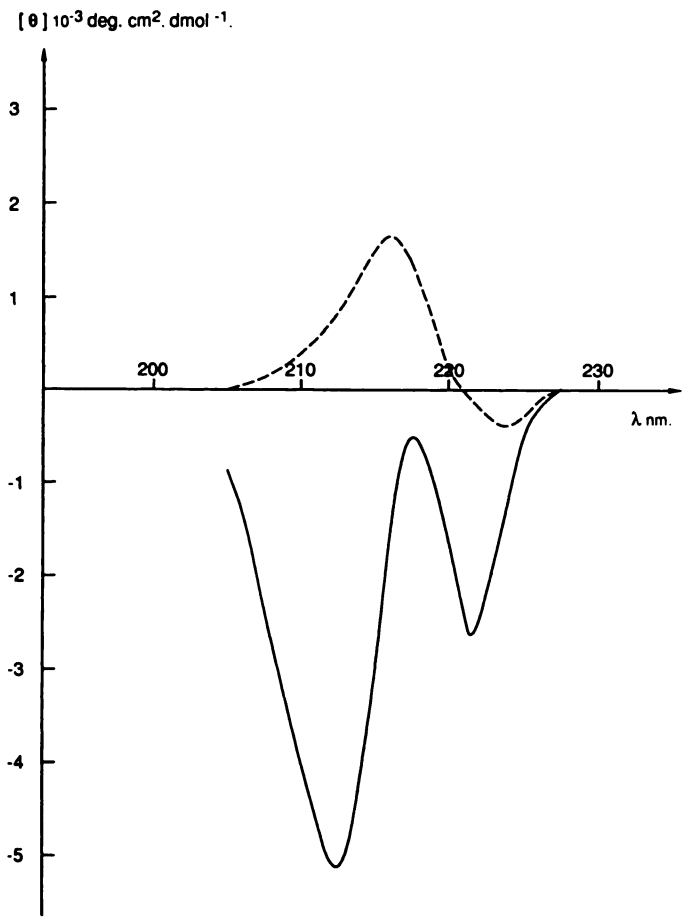

Fig. 1. CD spectra of VIP(1–11) (——) and VIP(7–11) (– – –) in the 200–250-nm region.

gen-bonded NH in this peptide has been shown by determination of the temperature coefficients $d\delta/dT$ for VIP(1–11) in DMSO-d_6 (Table 1), after proper assignment using COSY (Fig. 2). Values of more than -3×10^{-3} ppm/°C are indicative of solvent-shielded and presumably hydrogen-bonded NH groups; values of less than -4×10^{-3} ppm/°C are assigned to exposed groups (19). On the basis of these assumptions and the experimental data (Table 1), it can be assumed that the amide protons of Ala[4], Val[5], and Tyr[10] are involved in intramolecular hydrogen bonds.

These results revealed an interaction between the three residues inducing a semirigid conformation in VIP(1–11). This can be accounted for by the existence of a type I or III β-turn structure stabilized by a hydrogen bond between the proton amide of Val[5] and the CO of the residue Ser[2] and an additional hydrogen bond between Ala[4]-NH and the OH of the Ser[2] side chain. This particular binding between the side chain of the i-th residue, which might be Ser, Thr, or Asx, and the main chain NH of the $(i + 2)$-th residue is referred to as an Asx turn (20). This was suggested for other peptides, like the Ser-Pro-Lys-Lys sequence (21). The second β turn would be type II and stabilized by a hydrogen bond between Tyr[10] NH and Thr[7] CO.

Competitive binding studies. Epithelial cells were incubated with increasing concentrations of ^{125}I-VIP. Available binding sites for the radioligand were saturable and specific. Equilibrium binding occurred after 40–60 min at 37° and remained constant for up to 120 min. Specific binding was 60–70% of total ^{125}I-VIP bound, which represents 6–8% of the total

TABLE 1

Chemical shifts, coupling constants, temperature coefficients of amide protons, and $^3J_{NHC_\alpha H}$ coupling constants of the endecapeptide

Residue	Chemical shifts				$^3J_{NHC_\alpha H}$[a]	$\Delta\delta/\Delta T$
	NH	Hα	Hββ′	Others		
	δ ppm				Hz	ppb/°C
His	b	4.65	2.80	7.34		
				6.91		
Ser	8.67	4.30	3.63		b	−5.2
Asp	8.10	4.57	2.69		5.60	−3.8
			2.52			
Ala	7.75	4.27	1.14		6.05	−2.7
Val	7.65	4.06	1.88	0.75	b	−0.6
Phe	8.03	4.65	3.07	7.15	8.10	−4.4
			2.79			
Thr	7.86	4.09	4.09	1.07	7.85	−5.2
Asp	8.07	4.63	2.70		b	−8.8
			2.51			
Asn	7.97	4.48	2.51		7.50	−3.8
			2.33			
Tyr	7.84	4.43	2.96	7.03	7.45	−3.4
			2.70	6.62		
Thr	7.65	4.20	4.00	1.02	b	−4.4

[a] Coupling constants are given in absolute values.
[b] Broadened signal.

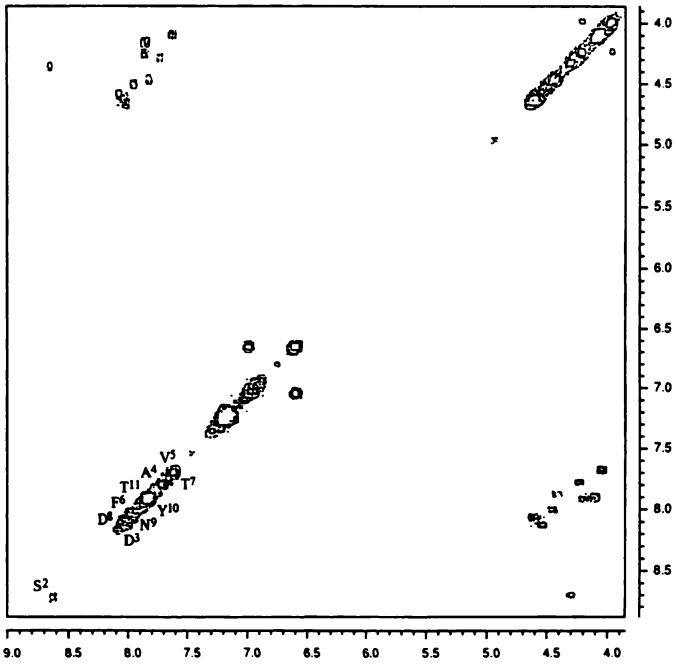

Fig. 2. Low field region of a 400-MHz COSY spectrum of the 11-residue segment His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr.

radioactivity added to the medium. The Scatchard plot for ^{125}I-VIP binding at equilibrium gave a curvilinear curve with a upward concavity over the range of 1–300 pM (Fig. 3). These data were resolved into two straight lines, suggesting the presence of two functionally different classes of binding sites. Binding parameters were $K_{d_H} = 0.034$ nM, $B_{max_H} = 36$ fmol/mg of protein, $K_{d_L} = 3.019$ nM, and $B_{max_L} = 377$ fmol/mg of protein, for the high and low affinity binding components, respectively.

Moreover, as shown in Fig. 4, VIP inhibited ^{125}I-VIP binding, and the IC_{50} value was 4.37 nM. The experiment examining competition of ^{125}I-VIP by VIP(1–11) showed that the 1–11 fragment was able to recognize only 35% of VIP sites (Fig. 4). From these data and the Scatchard plot (Fig. 3), we can

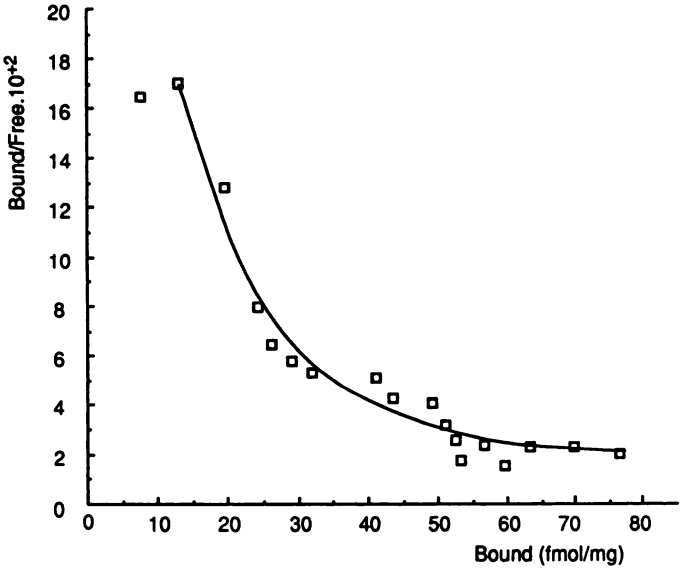

Fig. 3. Scatchard plot of ^{125}I-VIP binding to epithelial cells. The concentrations of ^{125}I-VIP were in the range of 1–300 pM, and the incubation time was 60 min. Individual points were the mean of two measurements.

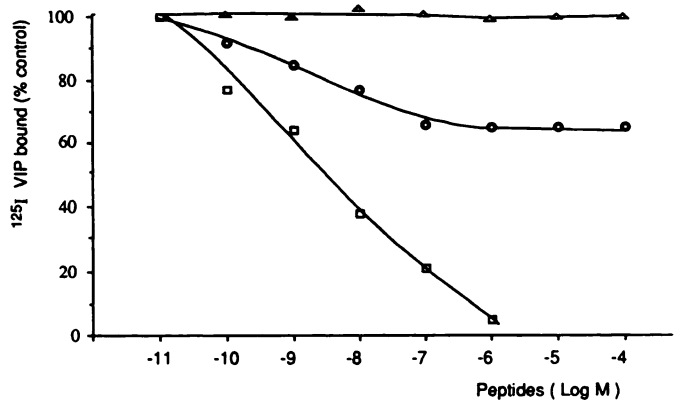

Fig. 4. Effects of VIP(1–11) on inhibition of binding of labeled ^{125}I-VIP to epithelial cells. Cells were incubated for 60 min at 37° with 100 pM ^{125}I-VIP plus various concentrations of VIP (□), VIP(1–11) (○), or VIP(10–28) (△). Specific binding was 7 ± 1% of the total radioactivity present in the sample.

conclude that there is possible VIP receptor heterogeneity in epithelial cells. In addition, any displacement of VIP by the 10–28 fragment is noteworthy.

This study is the first evidence for receptors of VIP on guinea pig tracheal epithelial cells. Such receptors have been described in two types of lung cells (22, 23) and colonic epithelial cells (8), with K_d values of 0.27 nM, 0.3 nM, and 0.13 nM and B_{max} values of 11.2 fmol/mg, 800 fmol/mg, and 67 fmol/mg, respectively.

Tracheal smooth muscle responses. We first studied the activity of VIP on trachea either precontracted or not with 0.1 μM carbachol, the concentration producing 50% of the maximum contraction. Cumulative addition of VIP, giving concentrations within the range of 0.1–1000 nM, produced a concentration-dependent relaxation of the trachea (Fig. 5). This result shows that the relaxation can be observed without precontraction, in contrast to relaxation in the ferret (24).

The role of the epithelial layer was analyzed by its total removal. Fig. 6 shows that differences were observed between intact and rubbed trachea in VIP-induced concentration-response curves. Significant differences have been noted for nonprecontracted trachea. In rubbed preparations, a 2 times greater relaxing response was observed (in the range of 1–50 nM) when the same VIP concentration was applied to each trachea. EC_{50} values (concentration of agonist producing 50% of the maximum response) were 3.83 ± 1.05 nM and 16.25 ± 5.35 nM in the absence and in the presence of epithelium, respectively. This result could be explained by the capacity of this tissue to synthesize some contracting and relaxing products from arachidonic acid (7), i.e., contracting substances derived from the lipoxygenase pathway, particularly leukotriene B_4, and relaxing substances derived from the cyclooxygenase pathway, like prostaglandin E_2. An altered epithelium would lose

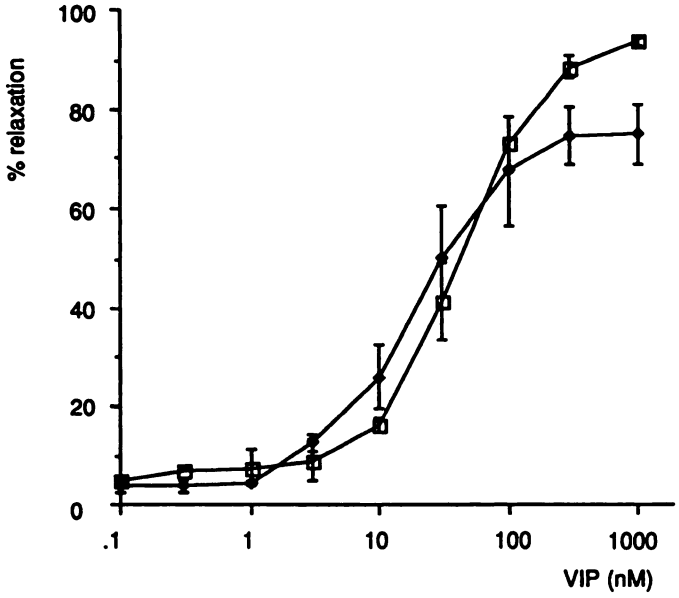

Fig. 5. VIP concentration-response curves in guinea pig trachea strips precontracted (□) or not (♦) by carbachol (10^{-7} M).

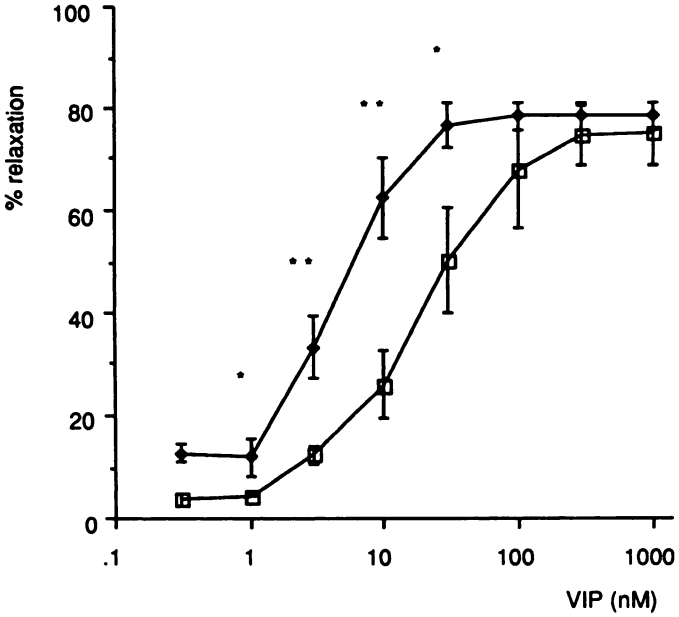

Fig. 6. VIP concentration-response curves in intact (□) and epithelium-free (♦) guinea pig tracheal strips. Values were means ± standard errors for three paired experiments. EC_{50} values were 3.83 ± 1.05 × 10^{-9} M (♦) and 16.25 ± 5.35 × 10^{-9} M (□). * and **, Values significantly different in the absence of epithelium (*, $p < 0.02$; **, $p < 0.01$).

its capacity to form such substances and this tonic regulation of the smooth muscle would be removed. In addition, the presence of epithelial enzymes can interfere with this epithelium regulation (25). This phenomenon was described for the degradation of tachykinins by metalloproteases secreted by tracheal epithelium (26).

The EC_{50} value (in the presence of epithelium), which reflects tracheal cell affinity for VIP, is ~4-fold higher than the IC_{50} obtained on epithelial cells. This difference was surprising and suggested a greater VIP affinity for epithelial cells than for all tracheal cells. This result supports the hypothesis of the importance of the epithelial layer in VIP-induced relaxation. The presence of other species that have VIP receptors but are less active or not active in the smooth muscle response could be involved in this discrepancy. The release of VIP fragments, like VIP(1–11), could explain this difference if the tracheal cells were able to cleave the VIP molecule. Recently, Sakai *et al.* (27) have reported that rabbit tracheal epithelium was able to modulate the VIP-stimulated ciliary motility by release of neutral endopeptidase. This sensitive method showed that the dose-response curves for VIP were shifted to lower concentrations by 0.5 log unit. During experimental times, relaxation curves did not show any significant shift, with or without inhibitors. So, it is noteworthy that the discrepancy observed between EC_{50} and IC_{50} values cannot be explained by VIP cleavage. A high performance liquid chromatography study showed that the relaxation medium did not contain proteolysis fragments of VIP, even in the absence of inhibitors. In each case, we could observe only 10% VIP degradation. So, a same low degradation rate may have a much more important effect on ciliary vibration than on tracheal relaxation.

VIP(1–11) at 10 μM induced a very slight relaxation of the intact tracheal strips, as previously described for VIP(1–10) on guinea pig trachea (28), but at concentrations greater than 10 μM the 11-amino acid sequence inhibited VIP-induced relaxation (Fig. 7). Ten minutes after VIP(1–11) addition, 0.1 μM VIP was added in the organ bath and the expected concentration-dependent relaxation decrease was observed. No similar effect could be observed for 10 μM VIP(10–28).

[D-*p*-Cl-Phe^6,Leu^{17}]VIP (29) has been tested under the same conditions, and an antagonistic effect has been observed for concentrations up to 1 μM.

Discussion

This work shows, for the first time, the presence of VIP receptors on guinea pig tracheal epithelial cells. Moreover, a precise evaluation of the VIP(1–11) affinity for specific sites should allow a better understanding of the VIP recognition process. This fragment seems to act as a competitive antagonist of VIP in the tracheobronchial tree; it partially inhibits specific ^{125}I-VIP binding to tracheal epithelial cells and VIP-induced relaxation of smooth muscle. Under the same experimental conditions, the 10–28 fragment did not exhibit any inhibition. The antagonist [D-*p*-Cl-Phe^6,Leu^{17}]VIP could be a good reference to test this effect. The results reported above were in favor of a heterogeneity of sites on the cell surface. VIP(1–11) was able to displace ^{125}I-VIP fixed on one type of receptors but could not be recognized by all the sites. In this respect, VIP might have two separate regions that interact with the binding area on the receptor, a feature suggested by structural and experimental data revealing that Tyr^{22} and Leu^{23} are involved in the binding process (30, 31). The heterogeneity reported above could be extended to the multiplicity of VIP receptors in different tissues. For example, the 10–28 fragment distinguishes VIP receptors from colon carcinoma cell line (32), spinal cord cells (33), lymphocytes (33), and tracheal epithelial cells.

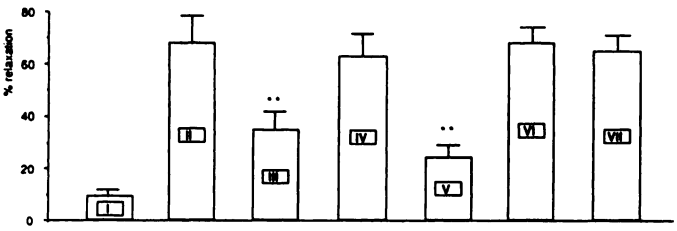

Fig. 7. VIP(1–11) (10^{-5} M) (*I*)- and VIP (10^{-7} M) (*II*)-induced relaxation. Competitive effect on VIP-induced (10^{-7} M) relaxation of VIP(1–11) (*III*, 10^{-5} M; *IV*, 5×10^{-6} M), [D-*p*-Cl-Phe^6,Leu^{17}]VIP (*V*, 10^{-6} M; *VI*, 10^{-7} M), and VIP(10–28) (*VII*, 10^{-5} M). **, Values significantly different for relaxation inhibition, $p < 0.01$.

Experimental studies in methanol, hexafluoroisopropanol, trifluoroethanol, or DMSO previously pointed out the presence of a helix at the Ct (18, 34), whereas the structures of the residues 1–4 and 7–10 segments of the molecule could not be clearly defined. The present work focusing on VIP(1–11), using either CD or NMR, shows that this fragment, dissolved in DMSO, exhibits two β-turn structures, one of them being probably stabilized by the presence of an Asx turn. According to our NMR study, the second β-turn is not as well defined as the residue 1–4 one. These results allow the hypothesis that the β-turn involving residues 7–10 could serve as an initiation site for a α-helix that, as proposed by Fournier *et al.* (18), modulates the overall conformation of VIP. This is in agreement with the work of Bodanszky *et al.* (34), using ORD-CD spectra and showing that the helical character of VIP in water and trifluoroethanol is still discernible in the docosapeptide VIP(7–28) but decreases with decreasing chain length. A helical structure for residues 11–23 is, however, suggested by most secondary structure algorithms used here.

VIP(1–11) displays antagonistic properties in inhibiting the VIP-induced tracheal relaxation. The effect of VIP(1–11) shown here is an important feature to improve knowledge of VIP action. A VIP deficiency was suspected in some malignancies (35), and an additional explanation of physiological disorders could be peptidolysis of VIP-releasing fragments, which would have antagonistic effects. At the present time, *in vivo* degradation of VIP is not clearly defined, and a hypothetical release of VIP(1–11) has not been characterized either *in vitro* or *in vivo*. Several authors have examined preferential cleavages from VIP by some enzymatic systems (27, 36, 37). The present contribution revealed that proteolysis of VIP with cathepsin G or elastase (from human leukocytes), two enzymes closely related to pulmonary inflammation, did not produce the residue 1–11 fragment. Future studies will investigate whether VIP(1–11) may be generated by specific enzymes.

Because of the heterogeneity discussed above, it can be expected that the antagonistic effect of VIP(1–11) may not be ubiquitous. In fact, the heterogeneity of VIP receptors that exists, in terms of pharmacology and interpretation of the peptide effects described by numerous studies, is probably connected to different recognition sites.

Among the binding sites in tissues, receptors in immune systems were recently characterized (38, 39) and, interestingly enough, a strong homology has been observed between the five-amino acid sequence of position 7–11 and a five-amino acid

sequence of the human immunodeficiency virus external glycoprotein molecule (gp 120), which is responsible for viral binding to the T lymphocytes (6, 40, 41). Other binding sites could be involved in regulation of superoxide anion production from human monocytes (42) or in singlet oxygen quenching (43). Lastly, adenylate cyclase-coupled receptors are certainly involved in mitosis and cell differentiation regulation, especially of sympathetic neuroblasts (44). This transcription pathway could be the means by which VIP causes *in vitro* differentiation of human neuroblastoma cells (45). It will be interesting to examine whether VIP(1–11) can compete with VIP in other activities, particularly in the latter property. Essential data about a mechanism of recognition between VIP and dedifferentiated cells would be provided.

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