Vasoactive Intestinal Peptide in Human Nasal Mucosa

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Abstract

Vasoactive intestinal peptide (VIP), which is present with acetylcholine in parasympathetic nerve fibers, may have important regulatory functions in mucous membranes. The potential roles for VIP in human nasal mucosa were studied using an integrated approach. The VIP content of human nasal mucosa was determined to be 2.84±0.47 pmol/g wet weight (n = 8) by RIA. VIP-immunoreactive nerve fibers were found to be most concentrated in submucosal glands adjacent to serous and mucous cells. ^{125}I-VIP binding sites were located on submucosal glands, epithelial cells, and arterioles. In short-term explant culture, VIP stimulated lactoferrin release from serous cells but did not stimulate [^{3}H]glucosamine-labeled respiratory glycoconjugate secretion. Methacholine was more potent than VIP, and methacholine stimulated both lactoferrin and respiratory glycoconjugate release. The addition of VIP plus methacholine to explants resulted in additive increases in lactoferrin release. Based upon the autoradiographic distribution of ^{125}I-VIP binding sites and the effects on explants, VIP derived from parasympathetic nerve fibers may function in the regulation of serous cell secretion in human nasal mucosa. VIP may also participate in the regulation of vasomotor tone. (*J. Clin. Invest.* 1990. 86:825–831.) Key words: respiratory glycoconjugates • mucus • lactoferrin • parasympathetic nervous system

Introduction

Vasoactive intestinal peptide (VIP;[1] 28 amino acid residues; 1) is a neurotransmitter in postganglionic parasympathetic neurons. Preganglionic parasympathetic nerves of the nasal mucosa originate in the superior salivatory nucleus of the seventh cranial nerve, pass through the Vidian nerve, and synapse with postganglionic cell bodies in the sphenopalatine ganglion (2–4). Postganglionic cholinergic neurons contain VIP, peptide with histidine at the NH_2 terminus and methionine at the COOH terminus (PHM), and both choline acetyl transferase and acetylcholinesterase, which indicate the presence of acetylcholine (5–7). The neurons enter the mucosa via the posterior nasal nerves and innervate submucosal glands, arterioles, and venules (3, 4, 7). Stimulation of parasympathetic neurons leads to the release of acetylcholine, which acts upon muscarinic receptors on submucosal glands (8) and possibly vessels (9, 10).

In humans, stimulation of submucosal glands by exogenous methacholine (8) or central reflexes (11) leads to secretion of such mucous cell products as mucous glycoconjugates, and serous cell products such as lactoferrin, lysozyme, secretory component, and secretory IgA (8, 11–13). Stimulation of vessels by either cholinergic agonists or central parasympathetic reflexes has been suggested to cause vasodilation (9, 10, 14, 15) and may contribute to the secretion of plasma products such as albumin and IgG. Parasympathetic efferent reflexes may be initiated by afferent sensory nerve stimulation (11, 13), or by "short circuit" stimulation of postganglionic parasympathetic cell bodies by nociceptive, substance P-containing trigeminal neurons (16). In addition, electrical stimulation of the sphenopalatine ganglion leads to atropine inhibitable nasal secretion and atropine-resistant vasodilation (3).

Since VIP is released along with acetylcholine by parasympathetic nerves, VIP may play an important role in regulating nasal responses. However, there is little understanding of the functions of VIP in the nasal mucosa. Therefore, the concentration of VIP in human nasal mucosa was measured by RIA, the locations of VIP-immunoreactive nerve fibers were determined by immunohistochemistry, the distribution of ^{125}I-VIP binding sites was determined by autoradiography, and the effects on the secretion of mucous and serous cell products from submucosal glands were determined by exposing human nasal mucosal fragments to VIP in short term explant culture. Based upon this integrated approach, inferences were made about the role of VIP in the regulation of vascular and glandular responses of human nasal mucosa.

Methods

Tissue handling. Human inferior turbinates were obtained at the time of surgery from 35 patients with nasal obstructive syndromes. No patient had had a recent infection. At the time of surgery, 2% tetracaine HCl and 0.25% phenylephrine HCl were applied topically on nasal packs. The turbinates were injected with 2–4 ml of 1% lidocaine with 1:100,000 epinephrine. An incision was made from the lateral wall of the inferior turbinate at the level of the infundibulum through the inferior conchal bone, and then inferiorly along the medial aspect of that bone. The medial flap of turbinate that remained was wrapped superiorly and laterally to close the wound. Within 20 min of surgical excision, the nasal mucosa was dissected from the inferior conchal bone. Mucosal specimens for autoradiography were frozen in 2-methyl-butane on dry ice for 20 s, then stored at −70°C. Specimens for explant culture were placed in L15 media (Biofluids, Rockville, MD) supplemented with 100 μg/ml penicillin, 100 μg/ml streptomycin, and 0.5 μg/ml amphotericin for transport to the laboratory.

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1. *Abbreviations used in this paper:* P1, period 1; P2, period 2; PHM, peptide with histidine at the NH_2 terminus and methionine at the COOH terminus; VIP, vasoactive intestinal peptide.

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Tissue fixation. Fresh tissues for immunohistological examination were microwave fixed. Individual 5 × 2 mm fragments of tissue were placed in plastic scintillation vials (Kimble, Toledo, Ohio) with 10 ml of 1.5% paraformaldhyde and 0.05% glutaraldehyde in pH 7.4, 50 mM sodium phosphate, 0.1 M NaCl (PBS) at room temperature and microwaved (400 W, Sharp Instruments, Mahwah, NJ) for 5 s (17). The temperature of the solution was raised to 45±5°C. After fixation, samples were stored at 4°C before being embedded in paraffin (American Histolabs, Gaithersburg, MD).

Turbinate extract preparation for RIA. Frozen turbinate tissue from single individuals was weighed (wet weight) and the tissue finely dissected with razor blades. Three extract procedures were contrasted.

(*a*) Boiling 0.1 N acetic acid in distilled water was added (20 μl/mg tissue) and boiled for 30 min (adapted from reference 18). During these 30 min, the preparation was homogenized in a ground glass tissue homogenizer, and sonicated at three intervals (Heat Systems-Ultrasonics, Inc., Plainview, NY) for 30 s each at a setting of 6. The suspension was centrifuged (1,700 *g*, 30 min, 4°C) and the supernatant was lyophilized.

(*b*) A solution of cold 50% ethanol, 50% 0.1 N acetic acid, 0.02% sodium bisulfite in distilled water was added at 20 μl/mg tissue, and chilled at 4°C for 20 min (adapted from references 19 and 20). During this period, the mixture sonicated three times for 30 s each on ice. The tubes were centrifuged and the supernatant was lyophilized.

(*c*) 2 ml of acetone at 4°C were added to 100–300 mg of turbinate tissue and sonicated as above (adapted from reference 21). After centrifugation, the supernatant was dried by Speed-Vac (Savant Industries, Hicksville, NY).

The powdered extracts were resuspended in RIA buffer (0.1% BSA, pH 7.4, 0.1 M sodium phosphate, 0.05 M NaCl, 0.01% NaN_3, 0.01% Tween-80) so that powdered extract equivalent to 10 mg of original tissue mass was suspended in 100 μl of RIA buffer.

RIA. RIA reagents were purchased from Peninsula Laboratories (Belmont, CA) and included 3,326 g/mol VIP, 700 Ci/mmol ^{125}I-tyr-VIP, polyclonal rabbit antiserum to VIP, goat anti-rabbit gamma globulin serum, and normal rabbit serum. Standard peptide solutions were prepared for the range from 0.15 to 77.0 fmol per tube (0.5–256 pg per tube). As a control, samples of ethanol-acetic acid extracts of human nasal mucosa equivalent to 5 mg of tissue were also added to standard amounts of VIP to determine if the addition of tissue extracts affected the accuracy of the RIA.

Turbinate extracts were diluted and aliquotted to give extracts equal to 10, 3, and 1 mg of turbinate tissue per tube. Rabbit anti-VIP serum was added. After overnight incubation at 4°C, ^{125}I-VIP was added. After a second overnight incubation at 4°C, polyclonal goat anti-rabbit gamma globulin and nonimmune rabbit serum were added for 2 h at room temperature. RIA buffer was added, the tubes centrifuged at 1,700 *g* for 40 min, and the supernatants aspirated. The pellets were counted in a gamma scintillation counter (Beckman Instruments, Irvine, CA) and the percentage of bound to total counts (B/B_0%) was determined.

The linear portion of the standard curve was analyzed by linear regression and the yield of VIP per tube was interpolated. The femtomoles of VIP per tube and picomoles per gram turbinate tissue were calculated. The mean±SEM picomoles per gram turbinate tissue for each extraction method was determined.

HPLC. Nasal tissue was extracted in ethanol-acetic acid, lyophilized, and reconstituted in eluent A (0.12% trifluoroacetic acid; Sigma Chemical Co., St. Louis, MO). After 30 min, nonsoluble material was removed by centrifugation. An 150-μl aliquot was applied to a high performance liquid chromatogram (114M pumps, 421 controller, and 164 detector; Beckman Instruments, Inc., Fullerton, CA) using a C-18 reverse-phase column (ODS Ultrasphere, 4.6 × 25 cm, 5 μm pore size, Beckman Instruments, Inc.). Samples were eluted 5 min after injection using 45-min runs and a linear gradient of 0–90% eluent B (0.12% trifluoroacetic acid in acetonitrile) in eluent A (22). Synthetic VIP (Peninsula Laboratories) was used to standardize the column. Fractions were collected at 1-min intervals, frozen, lyophilized, and reconstituted in RIA buffer. The VIP content of each fraction was determined by RIA.

Indirect immunohistochemistry. 6-μm-thick paraffin sections were sequentially placed in xylene, graded alcohols, distilled water, PBS, and PBS with 1% nonimmune goat serum (11, 13). Sections were incubated with rabbit antisera (1:1,000) to VIP (Peninsula Laboratories) or with nonimmune rabbit serum for 18 or 44 h at 4°C. The slides were washed in PBS, reblocked with 1% nonimmune goat serum in PBS for 3 min at room temperature, and then 1:40 colloidal gold–labeled goat anti–rabbit gamma globulin (Auroprobe; Janssen Pharmaceuticals Piscataway, NJ) was added for 60 min at room temperature. After being washed three times in PBS for 5 min each and three times in distilled water for 3 min each at room temperature, silver enhancing solution (IntenSE; Janssen Pharmaceuticals) was added and the development of the stained structures was monitored by light microscopy. The slides were washed in distilled water, dehydrated in graded alcohols, and coverslips applied with Permount (Sigma Chemical Co.).

Autoradiography. Frozen sections of tissue were warmed to room temperature, incubated in pH 7.4, 50 mM Tris, 3 mM $MgCl_2$ for 10 min at 25°C, then incubated with 1 nM ^{125}I-VIP in pH 7.4, 50 mM Tris, 5 mM $MgCl_2$, 2% polypep (Sigma Chemical Co.), 1 μM thiorphan, 1 μM phosphoramidon, 40 mg/liter bacitracin, 5 mg/liter chymostatin, and 4 mg/liter leupeptin for 3 h at 37°C (adapted from reference 23). Nonspecific binding was determined by adding 1 μM VIP to the incubation mixture. After incubation the slides were washed twice in pH 7.4, 50 mM Tris for 5 min each at 4°C. The slides were dried with a stream of cold dry air.

Nuclear track emulsion (NTB-2; Eastman Kodak Co., Rochester, NY) was melted in the dark for 4 h at 45°C and then mixed with 1% glycerol in water. In a darkroom, slides were dipped in emulsion, the backs of the slides wiped clean, and the slides placed vertically for 2 h to allow the emulsion to run off in an even fashion and dry (24). Coated slides were placed in plastic boxes with hygroscopic calcium sulfate (W. A. Hammond Drieite Co., Xenia, OH) and stored at −20°C.

Slides were removed at intervals and warmed to room temperature in the dark. They were developed in D-19 developer (Eastman Kodak Co.) for 2 min at 22°C, washed in water for 30 s, fixed in Kodak fixer for 4 min, and finally washed in water for 15 min.

Human nasal mucosal explant culture. To quantify the release of secretory cell products in response to VIP, fresh human nasal mucosa was cut into 3 × 3 mm fragments (25, 26). Pairs of fragments were placed on gelfoam in petri dishes, and cultured in 2 ml CMRL 1066 containing 100 μg/ml penicillin, 100 μg/ml streptomycin, 0.5 μg/ml amphotericin (Gibco Laboratories, Grand Island, NY), and 1 μCi/ml [^{3}H]glucosamine (New England Nuclear, Boston, MA). The [^{3}H]glucosamine becomes incorporated into newly synthesized respiratory glycoconjugates (25, 26). After 24 h, the media was changed and 400 KIU/ml aprotinin (Sigma Chemical Co.) was added to the mixture. After an additional 24 h, fresh media with aprotinin was added for 4 h (period 1 [P1]). The culture supernatant from this baseline period was collected and then replenished for 1 hour (period 2 [P2]) by aprotinin containing media with 0.1, 1, and 5 μM VIP (Peninsula Laboratories), 1, 10, and 100 μM methacholine (Sigma Chemical Co.); combinations of VIP and methacholine; or media (control plates). Supernatants from P1 and P2 were used for quantitation of respiratory glycoconjugates and lactoferrin.

Respiratory glycoconjugate release was quantified by precipitation of [^{3}H]glycoconjugates in 10% TCA and 1% phosphotungstic acid (TCA/PTA) at 5°C overnight. The precipitates were pelleted by centrifugation (1,200 *g* for 10 min), washed twice with TCA/PTA and hydrolyzed in 0.1 M NaOH. Aliquots of the resuspended precipitates were used for scintillation counting. The ratios of disintegrations per minute for P2 to P1 (secretory index) were calculated for each treatment. The secretory indices were compared with control cultures, and the percent change from control (mean±SEM) was calculated. Results were statistically compared to controls using the paired *t* test.

Lactoferrin ELISA. Quantitation of lactoferrin release into the culture media from P1 and P2 for each treatment was performed by using a noncompetitive ELISA (11). Microtiter plates were coated with 50 μl of rabbit anti-human lactoferrin (Dako Corp., Santa Barbara, CA) diluted 1:1,000 in 0.1 M carbonate buffer, pH 9.6, and incubated at 4°C overnight. The wells were washed with 4 vol of a buffer (PT) consisting of PBS, pH 7.4, with 0.05% Tween 80 (Fisher Scientific, Fair Lawn, NJ). After blocking nonspecific binding sites with 200 μl of 1% goat serum (Gibco Laboratories) in PT for 30 min at room temperature, 50 μl of media or standard (diluted in PT) was added to each well and incubated at 37°C for 90 min. Then 50 μl of goat anti-human lactoferrin conjugated to horseradish peroxidase (Organon Teknika-Cappel, West Chester, PA) was added and incubated at 37°C for 90 min. The color reaction was developed with an *o*-phenylenediamine dihydrochloride substrate (Sigma Chemical Co.), and then the optical densities of the plates were read at 490 nm on an ELISA reader (Dynatech Laboratories, Alexandria, VA). The ratios of optical densities from the supernatants collected during P2 to that for P1 (secretory index) were calculated for each treatment as above, and the mean (±SEM) percent change in secretory indices from control values was calculated. The secretory indices for each treatment were compared with the control values by paired *t* test.

Results

RIA. The sigmoid standard curve was linear between 1.2 and 38.5 fmol (4 and 128 pg) per tube. Using linear regression, the squares of the correlation coefficients for the standard curves were between 0.96 and 0.99. The sensitivity (concentration at B/B_0 = 50%) of the assay was 5.83±0.62 fmol per tube (n = 5). The addition of ethanol-acetic acid extract equivalent to 5 mg of nasal mucosa did not affect the shape of the standard curve or the slope of the linear portion, even though the addition did shift the curve slightly to the right. The VIP content of human turbinate nasal mucosa was estimated by interpolation of B/B_0% values from the linear portion of the standard curve.

Samples extracted with boiling acetic acid yielded 2.84±0.47 pmol per g wet weight tissue (n = 8). Content ranged from 1.07 to 4.61. Samples extracted with ethanol-acetic acid yielded 2.84±0.38 pmol per g (n = 17 patients) with a range from 0.70 to 5.80. Samples extracted with acetone yielded 0.80±0.10 pmol per g (n = 4), with a range between 0.23 and 1.03.

HPLC. Synthetic VIP (25 μg) eluted from the HPLC column with a single, narrow peak at 25.96 min. Using the RIA, VIP eluted only in fractions collected at 25 and 26 min. When turbinate tissue was eluted from the HPLC column, VIP immunoreactive material was collected only at 25 and 26 min. All other fractions for both the standards and tissue contained no immunoreactive VIP.

Indirect immunohistochemistry. Human inferior turbinate nasal mucosa is covered by ciliated pseudostratified columnar respiratory epithelium (Fig. 1). Beneath the basement membrane are fenestrated capillaries (27). Beneath this vascular

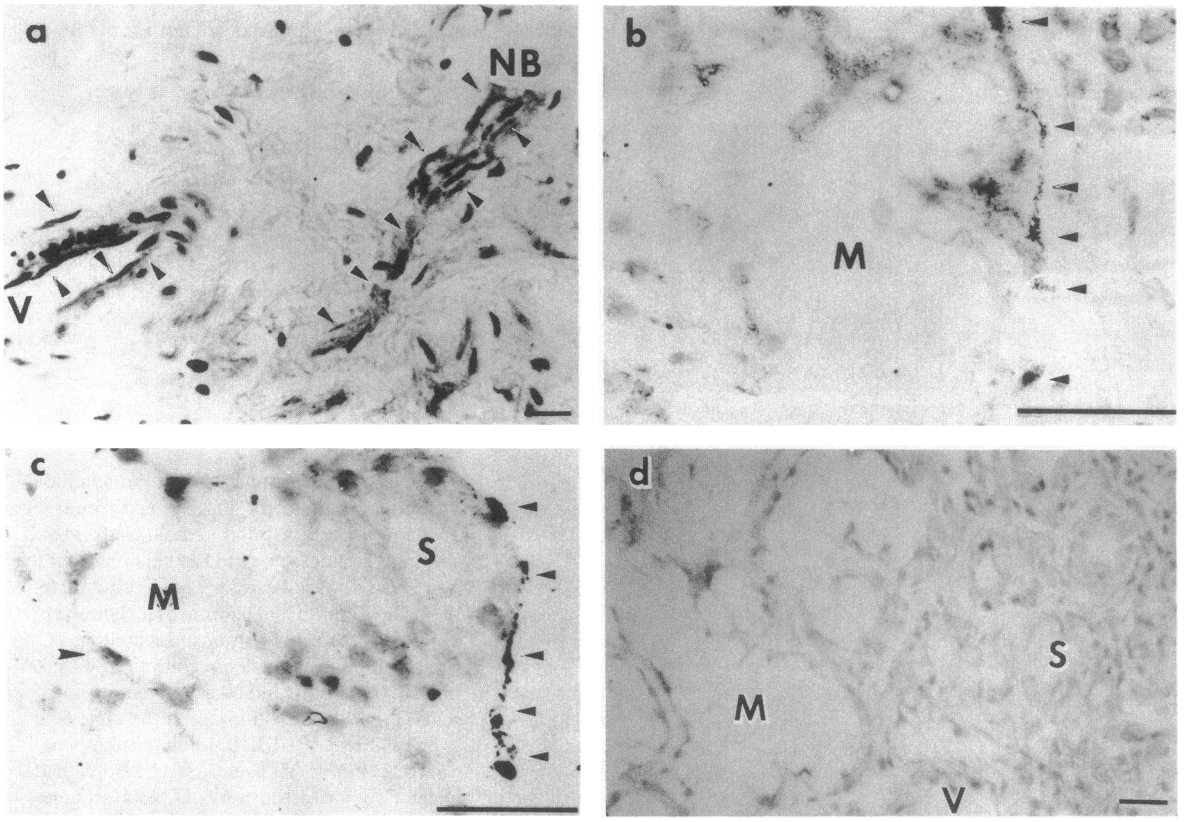

Figure 1. VIP immunoreactive nerve fibers in human nasal mucosa. (*a*) VIP nerve fibers (*arrowheads*) are seen in a nerve bundle (*NB*) and in the wall of a small venule (*V*). Hematoxylin counterstain. (*b*) A VIP nerve fiber (*arrowheads*) is seen innervating mucous cells (*M*) of a submucosal gland acinus. Nuclear fast red counterstain. (*c*) VIP nerve fibers are seen innervating serous cells (*S*) and mucous cells (*M*) of a submucosal gland. Nuclear fast red counterstain. (*d*) Negative control. VIP antiserum adsorbed with excess VIP did not identify VIP immunoreactive structures in these serous (*S*) and mucous (*M*) acinii or venule. Nuclear fast red counterstain. Bars, 25 μm.

zone are interspersed tubuloacinar seromucous submucosal glands and their ducts. In the connective tissue between submucosal glands are large venous sinusoids. Coiled arterioles and nerve bundles are located deeper in the mucosa.

VIP-immunoreactive material was identified readily in tissue fixed by microwave irradiation. Microwave fixation allowed rapid tissue processing and excellent preservation of tissue histology, and improved the identification of immunoreactive nerve fibers when compared with other fixation techniques (17). This may reflect the rapidity of tissue fixation by microwave irradiation.

Staining sections with nuclear fast red (Sigma Chemical Co.) allowed tissue definition which did not obscure nerve fibers. Other stains obscured detection of immunoreactive VIP nerves which could otherwise be seen in unstained sections.

VIP immunoreactive nerve fibers were most frequently encountered in submucosal glands (Fig. 1). The fibers were often in direct contact with acinar cells. There was apparent contact with both serous and mucous cells, and with the myoepithelial cells that surround each acinus. VIP fibers were seen in nerve bundles. Some VIP nerve fibers were also detected in the walls of arterial and venous vessels. More fibers were seen in glands than vessels. No fibers were found in the epithelium.

Autoradiography. ^{125}I-VIP binding sites on the epithelium and submucosal glands of human nasal mucosa were detected as deposits of silver grains using darkfield microscopy (Fig. 2). It was not possible to determine if ^{125}I-VIP bound preferentially to specific subsets of cells such as goblet cells, serous cells, or mucous cells since the use of mucous cell–specific stains such as alcian blue damaged the emulsion and dispersed the silver grains. There also appeared to be binding of ^{125}I-VIP to the vascular smooth muscle of arterioles (Fig. 3). It was not possible to differentiate between smooth muscle or endothelial binding of ^{125}I-VIP due to the scatter of radiation from ^{125}I decay. However, venous sinusoids bound very little ^{125}I-VIP, suggesting that the endothelium of these thin walled vessels had very few or no VIP binding sites. The addition of excess VIP ablated binding to epithelium, glands, and arterioles (Figs. 2 *f* and 3 *d*).

Human nasal mucosal explant culture. Methacholine (MC) between 1 and 100 μM induced dose dependant secretion of lactoferrin and [^{3}H]glucosamine labeled respiratory glycoconjugates (Table I). Release of lactoferrin was significantly stimulated at 1 μM MC whereas glycoconjugate release was unchanged from control, indicating that either lactoferrin containing serous cells responded to lower concentrations of methacholine than glycoconjugate containing cells, or that changes in lactoferrin secretion were more readily detected than changes in glycoconjugate secretion. VIP in doses of 0.1–5 μM induced significant lactoferrin secretion, but did not affect glycoconjugate secretion. At concentrations of 1 μM, methacholine was more potent than VIP at inducing lactoferrin secretion.

Since VIP and acetylcholine may be simultaneously released from parasympathetic nerves, the effects of combinations of methacholine and VIP were studied. The addition of VIP to 1 μM methacholine did not stimulate glycoconjugate release or augment the release of lactoferrin. The addition of VIP to 10 μM methacholine did not affect glycoconjugate release. Lactoferrin release was increased in additive but not

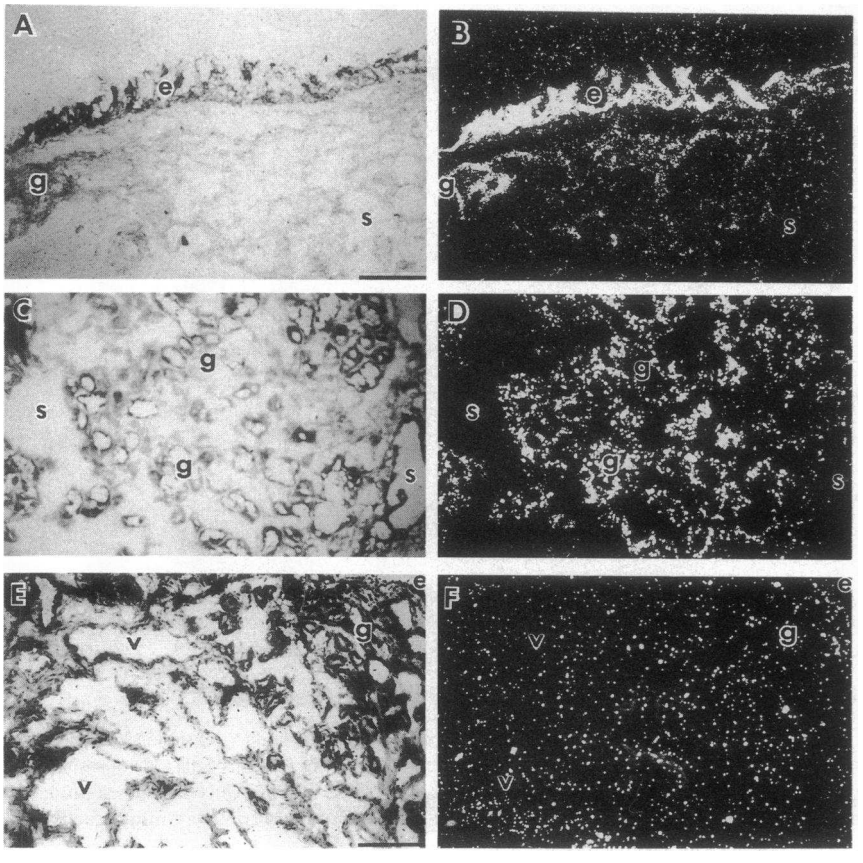

Figure 2. ^{125}I-VIP binding sites in human nasal mucosa. (*A*) Brightfield image of epithelium (*e*) and lamina propria. Submucosal glands (*g*) and sinusoids (*s*) are seen. (*B*) Darkfield image of the same field as *A*, indicating ^{125}I-VIP binding to the epithelium (*e*) and submucosal glands (*g*). Sinusoids (*s*) did not demonstrate binding. (*C*) Brightfield image of a dense collection of submucosal glands (*g*) and adjacent venous sinusoids (*s*). (*D*) Darkfield image of the same field as (*C*) showing ^{125}I-VIP binding to submucosal glands (*g*) but not sinusoids (*s*). (*E*) Brightfield image from a consecutively cut tissue section showing vessels (*v*) and submucosal glands (*g*) on a slide treated with 1 μM VIP. (*F*) Darkfield image of the same field as *E*, showing that the addition of excess VIP ablated the binding of ^{125}I-VIP. Toluidine blue counterstaining. Bars, 50 μm.

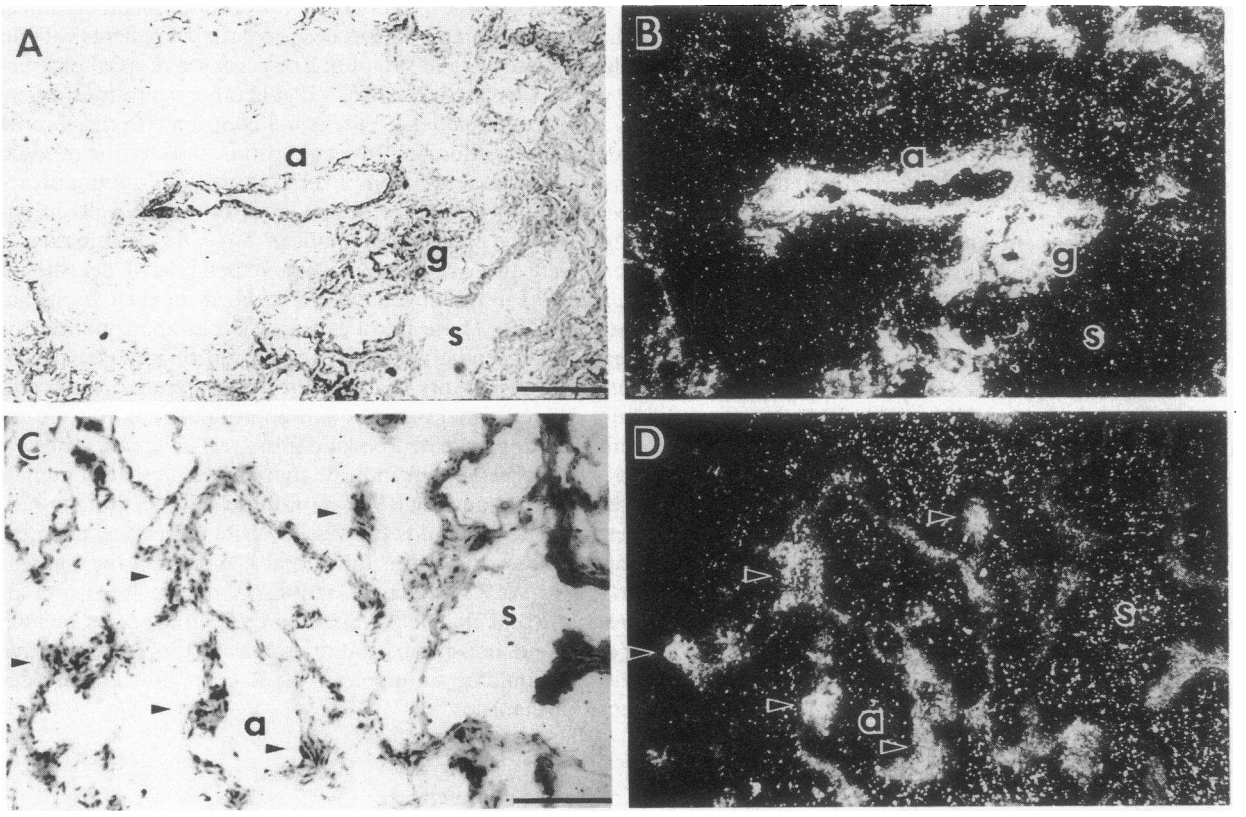

Figure 3. ^{125}I-VIP binding sites in human nasal mucosa. (*A*) Brightfield image of an arteriole (*a*), submucosal glands (*g*), and sinusoids (*s*). (*B*) Darkfield image of the same field as *A*, showing ^{125}I-VIP binding to the arteriole (*a*) and glands (*g*), but no binding to the venous structures (*s*). (*C*) Brightfield image showing several vessels with vascular smooth muscle in their walls (arrowheads indicate representative areas). Several arterioles (*a*), thin muscular veins, and venous sinusoids (*s*) are shown. (*D*) Darkfield image of the same field as *C* shows silver grains over the areas of vascular smooth muscle (*arrowheads*). Areas without smooth muscle did not appear to demonstrate binding. Toluidine blue counterstaining. Bars, 50 μm.

synergistic fashion by 5 μM VIP plus 10 μM methacholine. These results indicate that VIP stimulated serous cell secretion, but that VIP was less potent than methacholine. VIP did not potentiate the effects of methacholine on secretion from either serous cells or [^{3}H]glucosamine-labeled respiratory glycoconjugate-containing cells.

Table I. Effects of VIP and Methacholine on the Release of Lactoferrin and [^{3}H]Glucosamine-labeled Respiratory Glycoconjugates from Human Nasal Mucosa in Explant Culture

VIP	MC	*n*	Lactoferrin		Glycoconjugates	
μM	*μM*		*% Change*	*P*	*% Change*	*P*
0	100	16	460±96	<0.001	31.8±7.4	<0.001
0	10	7	563±177	<0.02	17.0±12.0	NS
0	1	6	124±24	<0.005	−0.6±2.6	NS
5	0	14	132±37	<0.01	3.9±5.1	NS
1	0	9	114±29	<0.01	−2.2±3.8	NS
0.1	0	9	90±35	<0.05	8.9±12.1	NS
5	10	9	759±320	<0.05	16.6±9.9	NS
1	10	7	574±203	<0.02	13.0±5.1	<0.02
0.1	10	6	411±125	<0.01	16.6±6.9	<0.01

Mean±SEM; unpaired *t* test.

Discussion

VIP was present in human nasal mucosa. The concentration of acid ethanol extractable VIP (2.84±0.38 pmol per g tissue) was slightly less than the concentration of neuropeptides which we have measured previously. Neuropeptide Y (NPY), a neurotransmitter of sympathetic neurons in human nasal mucosa (28, 29), has a concentration of 3.13±0.79 pmol per g tissue. The concentration of VIP was greater than the concentration of calcitonin gene-related peptide (CGRP; 0.54±0.08 pmol per g), a neurotransmitter of trigeminal, nociceptive, type C sensory neurons (16, 30–32). The relative enrichment of VIP reflects the abundant supply of parasympathetic, cholinergic nerves to the nasal mucosa.

VIP-immunoreactive nerve fibers were located predominantly around submucosal glands. Individual fibers were also seen in the walls of venules and arterioles. The distribution of VIP contrasts with that of CGRP (32) from sensory neurons, and NPY (29) of sympathetic neurons. CGRP fibers and NPY fibers densely innervate arterial vessels, whereas individual CGRP fibers and NPY fibers were present in the walls of venous vessels and occasionally in submucosal gland acini. CGRP fibers were also found in the nasal epithelium. VIP fibers in the trachea and bronchi have been found around blood vessels, in smooth muscle bundles, submucosal glands, and near the epithelium (33, 34).

^{125}I-VIP binding sites were found on submucosal glands, epithelium, and in the walls of arterioles and veins in human nasal mucosa. This was similar to the distribution of VIP found in the lower respiratory tract, where VIP receptors have been demonstrated on tracheobronchial epithelium, submucosal glands, and alveolar walls (23). The highest density of VIP receptors were found on pulmonary vascular smooth muscle, and bronchial smooth muscle of trachea and bronchi (35, 36).

VIP-containing neurons also contain a closely related peptide: PHM (37, 38). Although VIP, PHM, and acetylcholine are all present in the peripheral neurosecretory varicosities of postganglionic parasympathetic neurons (39), the amounts of each released during neural transmission appears to depend upon the nerve impulse frequency (39). At low rates, acetylcholine is selectively released. At high rates, acetylcholine together with VIP and PHM are released. VIP may augment the postsynaptic acetylcholine-induced secretory response in glands (e.g., cat salivary glands, reference 40), but may also have presynaptic inhibitory effects which could act to limit neuropeptide release. This mechanism would conserve the amount of stored peptides since there are no re-uptake mechanisms, and VIP and PHM can only be resupplied by axonal transport from the cell body (37, 39). This principle may be very important in understanding the physiology of parasympathetic neurons.

VIP stimulates many aspects of exocrine function and increases the content of chloride in intestinal and bronchial secretions, and pancreatic secretion of bicarbonate, water, and macromolecules (33). In respiratory tissues, however, the effects of VIP on glands have been inconsistent. Tracheal tissues from ferret (41, 42) and dog (43) release modest amounts of respiratory glycoconjugates ("mucus") in response to VIP. VIP induces serous cell exocytosis from ferret tracheal explants (42). Feline tracheal explants do not respond to VIP (44). However, isolated feline submucosal glands do respond (44), and VIP augments the secretory responses of methacholine (44) and other secretagogues (45). In humans, incubation of tracheal explants with 0.2–2,000 nM VIP inhibited [^{3}H]glycoconjugate release from normal subjects, but had no effect upon explants from subjects with chronic bronchitis (46).

The present explant results indicate that VIP stimulated lactoferrin secretion from serous cells. VIP was less potent than methacholine and did not significantly augment muscarinic receptor-mediated stimulation of lactoferrin secretion. VIP did not stimulate [^{3}H]glucosamine-labeled respiratory glycoconjugate release and did not affect methacholine-induced secretion. The release of lactoferrin was much more responsive than [^{3}H]glycoconjugate release. Either serous cells were more sensitive to methacholine than glycoconjugate-containing cells, or lactoferrin was a more sensitive marker of submucosal gland secretion than glycoconjugates. Therefore, VIP augments the effects of cholinergic stimulation by causing selective enrichment of serous cell products in nasal secretions. The activation of VIP receptors on serous cells of submucosal glands represents an additional component which must be considered when studying parasympathetic reflexes in human upper respiratory tract (11).

^{125}I-VIP binding sites were also found on vessels. VIP induces dilation of human submandibular arteries in vitro (15) and feline nasal vessels in vivo (47, 48). VIP augments the cutaneous plasma extravasation that can be induced by substance P (49).

Alterations of VIP metabolism may contribute to several clinical syndromes. Increased VIP immunohistochemical staining in nasal mucosa has been reported in patients with an allergic diathesis and symptoms of paroxysmal nasal obstruction (50). Increased release of VIP and other neurotransmitters by the parasympathetic route could contribute to the chronic vascular congestion and hypersecretion characteristic of some obstructive nasal syndromes. In contrast, VIP-immunoreactive nerve fibers may be absent from bronchial walls in patients who die of status asthmaticus (51). These asthmatic patients were also reported to have increased relative densities of substance P-immunoreactive nerve fibers in their bronchial mucosa (52). These findings suggest that abnormalities of neuropeptide containing nerves could contribute to the pathogenesis of respiratory diseases. Prospective studies with carefully defined patient groups and appropriate controls are required to implicate neuropeptides in specific disease states.

These observations indicate that VIP is present in human nasal mucosa and that VIP-immunoreactive neurons innervate submucosal glands and vessels. VIP binding sites are present on the epithelium, submucosal glands, and the walls of arterioles. VIP stimulated lactoferrin release from human nasal mucosal fragments. Since serous cell products include specific and nonspecific antimicrobial factors, this selective response could be an important regulator of mucosal host defense mechanisms.

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