

Effects of neuropeptides on neutrophil migration through noncellular and endothelial barriers

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Background: Neuropeptides and neutrophils are postulated to be important immune effector molecules and cells, respectively, in a variety of lung inflammatory conditions.

Methods: We examined the effects of three lung neuropeptides, substance P, vasoactive intestinal peptide (VIP), and somatostatin and two relatively protease-resistant peptides, helodermin and sandostatin, on human neutrophil migration across 3 μ m pore filters and human endothelial monolayers cultured on these filters.

Results: At concentrations of 1 to 10 μ mol/L, substance P induced significant neutrophil migration that was dose-responsive and equivalent through endothelium and filters. Neither VIP, helodermin, somatostatin, nor sandostatin induced significant neutrophil migration through either barrier at doses of 10^{-14} to 10^{-5} mol/L. Because VIP and somatostatin have been reported to inhibit some inflammatory cell functions, we also examined their effects on chemoattractant-induced neutrophil migration. Pretreatment of neutrophils and/or endothelium with VIP or somatostatin at either 10^{-5} or 10^{-10} mol/L did not significantly inhibit formyl-methionyl-leucyl-phenylalanine-, leukotriene B_4 -, or platelet activating factor-induced neutrophil migration through naked filters or endothelium. Thus only substance P had significant effects on neutrophil chemotaxis.

Conclusions: Substance P, but not VIP or somatostatin, may be important in directly influencing neutrophil migration across endothelium towards lung inflammatory sites. However, all three neuropeptides might still modulate neutrophil functions and lung inflammatory responses through effects on other lung cells. (*J ALLERGY CLIN IMMUNOL* 1993;92:589-98.)

Key words: Neutrophils, chemotaxis, neuropeptides, neurogenic inflammation

There is much evidence suggesting that neuropeptides play important roles in controlling lung functions in health and disease.¹⁻³ Neuropeptides are widely distributed in the respiratory tract and have a variety of potent physiologic actions including modulation of airway calibre, vascular tone, vascular permeability, and mucus secre-

Abbreviations used

BSA:	Bovine serum albumin
ECGS:	Endothelial cell growth supplement
EDTA:	Ethylenediaminetetraacetic acid
FMLP:	Formyl-methionyl-leucyl-phenylalanine
HBSS:	Hank's balanced salt solution
HUVE:	Human umbilical vein endothelial
LTB ₄ :	Leukotriene B ₄
NSM:	Net stimulated migration
PAF:	Platelet activating factor
PBS:	Phosphate-buffered saline
VIP:	Vasoactive intestinal peptide

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tion.¹⁻⁴ In addition, many neuropeptides are capable of affecting inflammatory cell functions. Indeed, through their direct effects on multiple cells including monocytes, mast cells, and polymorphonuclear leucocytes, neuropeptides might play a significant role in the pathogenesis of neurogenic inflammation.¹⁻¹² Thus neuropeptide-induced in-

flammatory cell responses have been postulated to be important in inflammatory lung diseases.^{8, 9}

Three lung neuropeptides, substance P, vasoactive intestinal peptide (VIP), and somatostatin, have been implicated as modulators of immune effector cell function and responses.^{1-3, 5, 12-25} However, there are conflicting reports on the chemotactic effects of these three neuropeptides. Substance P has been reported to bind to rabbit neutrophil formyl-methionyl-leucyl phenylalanine (FMLP) receptors¹⁵ and to be chemotactic for both rabbit¹⁵ and rat neutrophils.¹⁶ In contrast, substance P has been reported to be only chemokinetic and not chemotactic for human neutrophils.¹⁸ VIP has been reported to both inhibit and stimulate monocyte and neutrophil chemotaxis depending on the doses used.¹⁷ We therefore designed a series of experiments to systematically examine the effects of substance P, VIP, and somatostatin on chemotactic responses.

Because neutrophils play an important role in a variety of inflammatory lung conditions, we examined the effects of these three neuropeptides (substance P, VIP, and somatostatin) and two related, relatively protease resistant peptides (helodermin²⁶ and octreotide²⁷), on neutrophil chemotaxis through naked filters and human umbilical vein endothelial (HUVE) cell monolayers grown on filters. We also examined the ability of VIP and somatostatin to modulate chemoattractant-induced neutrophil migration through these barriers.

On the basis of reports of the effects of neuropeptides on neutrophil chemotactic processes,¹³⁻¹⁹ we postulated that: (1) substance P would indeed be chemotactic for neutrophils; and (2) VIP, somatostatin, and their related peptides, helodermin and sandostatin, might not directly mediate neutrophil chemotaxis but would inhibit neutrophil chemotaxis induced by inflammatory mediators.

METHODS

Reagents

FMLP, leukotriene B₄ (LTB₄), collagen (bovine placental type), Ficoll type 400 DL, endothelial cell growth supplement, heparin, substance P, phosphoramidon [N-(α (rhamnopyranosyloxyhydroxyphosphinyl)-L-leucyl-L-tryptophan], angiotensin converting enzyme inhibitor (p-Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Hypaque M-90% was a gift from Winthrop-Brean (New York, N.Y.). Chromium-51, Na₂CrO₄ (⁵¹Cr), was purchased from New England Nuclear Co. (Boston, Mass.). Ham's F12K, fetal

calf serum, trypsin, ethylenediaminetetraacetic acid (EDTA), antibiotics, phosphate-buffered saline, and Hank's balanced salt solution (HBSS) were purchased from the University of Iowa Cancer Center (Iowa City, Iowa). VIP, somatostatin, and helodermin were purchased from Peninsula Laboratories (Belmont, Calif.). Sandostatin was a kind gift from Sandoz Pharmaceuticals (East Hanover, N.J.). Transwell tissue culture plates were purchased from Costar (Cambridge, Mass.). Platelet activating factor (PAF) (C16:0) was purchased from Bachem Biosciences (Pittsburgh, Pa.).

Cell lines

HUVE cells (passage 13) were purchased from the American Type Culture Collection (Rockville, Md.).

Cell culture

HUVE cells were grown as monolayers in tissue culture flasks or dishes incubated in 100% humidity and 5% CO₂ at 37° C. HUVE cells were grown in Ham's F12K medium supplemented with fetal calf serum (20%), heparin (100 μ g/ml), endothelial cell growth supplement (50 μ g/ml), penicillin (20 U/ml), and streptomycin (20 μ g/ml).^{28, 29}

The cells from the monolayers were harvested with trypsin (0.25%) and EDTA (0.1%) in phosphate-buffered saline, centrifuged at low speed (250 g, 5 minutes), and resuspended in fresh medium before they were grown on permeable filters in Transwell tissue culture plates. The cells were grown to confluence on these filters in Transwell plates over 2 to 3 days under the same conditions as outlined above for the tissue culture dishes or flasks. Transwell plates have 24 wells, which are separated into upper and lower chambers by polycarbonate filters that have a diameter of 6.5 mm and a pore size of 3.0 μ m. Chemotactic experiments were done with the use of the Transwell plates. Culture flasks and filters for HUVE cell experiments were treated with collagen (50 μ g/ml) overnight followed by rinsing with HBSS.

Isolation of neutrophils and chromium labeling

Neutrophils were isolated from 0.2% EDTA-anticoagulated whole blood collected by venipuncture from normal donors. The use of blood cells for these studies was approved by the University of Iowa Institutional Review Board for Human Studies, and all blood donors signed an informed written consent form. Neutrophils were obtained with a modification of the density gradient technique (1.095 gm/ml Hypaque-Ficoll) described by Ferrante and Thong.³⁰ The isolated neutrophils were washed once with Ca/Mg-free HBSS. Neutrophils were then resuspended in 4 ml of Ca/Mg-free HBSS, and residual red cells were lysed with 1% ammonium oxalate. The neutrophils were then washed again in HBSS without Ca or Mg before cell counts and viability determinations were performed by the trypan blue

exclusion method. The isolated neutrophils were at least 98% pure and 99% viable.

Isolated neutrophils were labeled with ^{51}Cr before they were placed in the chemotactic chambers by means of a modification of the procedure described by Gallin et al.³¹ Neutrophils at concentrations of 15 to 45×10^6 cells/ml were incubated in Ca/Mg-free HBSS with 0.5 mCi of ^{51}Cr (5 mCi/ml) at 37°C for 1 hour with vigorous mixing. The labeling was terminated at the end of the incubation period by diluting the cells to 15 ml with Ca/Mg-free HBSS. The neutrophils were subsequently washed three times in HBSS before they were resuspended in HBSS with 0.2% BSA and Ca/Mg. Aliquots of the cell suspension were removed and assayed for total counts and unbound ^{51}Cr to determine the cell-associated counts per minute (cpm) as described by Gallin et al.³¹ All peptides were dissolved in HBSS with Ca/Mg at 10^{-3} mol/L, aliquoted, and stored at -70°C .

Chemotaxis and transmigration

Previous experiments demonstrated that phosphoramidon, angiotensin converting enzyme inhibitor, or the combination did not exhibit any effect on neutrophil chemotaxis through naked filters or HUVE monolayers. Consequently, all chemotactic agents, control blanks, and test neuropeptides were reconstituted in HBSS with Ca/Mg containing 10^{-5} mol/L phosphoramidon and 10^{-5} mol/L angiotensin converting enzyme inhibitor.

Neutrophil chemotaxis through filters alone and transmigration of neutrophils through cell monolayers cultured on filters were investigated with the use of the 24-well tissue-culture-treated Transwell plates as previously described.^{28, 29} Monolayers of HUVE cells were grown on collagen-coated Transwell filters. We have previously shown that the presence or absence of collagen or lysed cell monolayers on filters does not influence LTB_4 -stimulated neutrophil chemotactic responses.²⁸ All neutrophil chemotaxis and transmigration studies were done with a standardized protocol. Before each experiment involving cell monolayers, the media was removed from the wells, and the monolayers were extensively washed with HBSS containing 0.2% BSA. One to 1.5 million ^{51}Cr -labeled neutrophils in 100 μl of buffer were placed in the upper chamber, above the filter or monolayer. The compound under investigation in 500 μl of buffer was placed in the lower chamber (below the filter). A negative buffer (HBSS- 0.2% BSA) control was included for each experiment. Each variable was tested in triplicate or quadruplicate. The plates were incubated at 37°C in 5% CO_2 and 100% humidity for 3 hours. After incubation, the lower chamber contents (500 μl) were mixed with an equal volume of 2% (vol/vol) Triton X-100 in H_2O , collected, and counted in a gamma counter. The counts per minute of the samples were calculated as counts per minute per 10^6 cells according to the formula used by Gallin et al.³¹ The data were then ex-

pressed as the percent net stimulated migration (NSM) with the formula:

$$\frac{(\text{cpm of experimental sample}) - (\text{cpm of negative control sample})}{\text{Total cpm added to chamber}} \times 100\%$$

The inter- and intraassay coefficients of variation for neutrophil NSM were usually 10% to 15% . Counts per minute measured in the negative control (HBSS-BSA) samples were generally less than 2% of the total counts per minute added to the top chambers when there was an overlaying monolayer on the filter and less than 8% when there was not an overlaying monolayer.

Neutrophil and HUVE cell viability was consistently greater than 94% after 3 hours of incubation with buffer or up to 10 $\mu\text{mol/L}$ of a chemoattractant. Neutrophil-associated ^{51}Cr and specific activity of the neutrophils were greater than 95% after exposure to chemotactic stimuli for 3 hours.

Pretreatment of neutrophils and HUVE monolayers with VIP and somatostatin

VIP and somatostatin at concentrations of 10^{-5} and 10^{-10} mol/L were used to pretreat HUVE monolayers and/or neutrophils for 30 minutes at room temperature. The cells were then washed with HBSS with Ca/Mg. Subsequently, we examined the effects of these pretreatment regimens on neutrophil transendothelial migration induced by FMLP (10^{-10} mol/L), LTB_4 (10^{-9} mol/L), and PAF (10^{-7} to 10^{-8} mol/L). Dosages of chemoattractants were chosen on the basis of previous data.^{28, 29} Incubation time for chemotactic assays was 3 hours.

RESULTS

Initial experiments focused on substance P, and in Fig. 1, *A*, the neutrophil chemotactic response through filters and HUVE monolayers can be seen. No significant neutrophil migration through either barrier was observed at doses of substance P from 10^{-14} to 10^{-6} mol/L. However, there was significant neutrophil migration through both barriers at 10 $\mu\text{mol/L}$. On the basis of these findings, we next performed dose-response experiments with doses of substance P between 1 and 10 $\mu\text{mol/L}$. As shown in Fig. 1, *B*, substance P induced equivalent neutrophil migration through both naked filters and HUVE monolayers in dose-dependent fashion.

We subsequently examined the effects of VIP and its more stable analog, helodermin, on neutrophil chemotaxis. VIP had no neutrophil chemotactic effect through either barrier (Fig. 2, *A*) at concentrations between 10^{-14} and 10^{-5} mol/L. In Fig. 2, *B*, the dose response of helodermin-

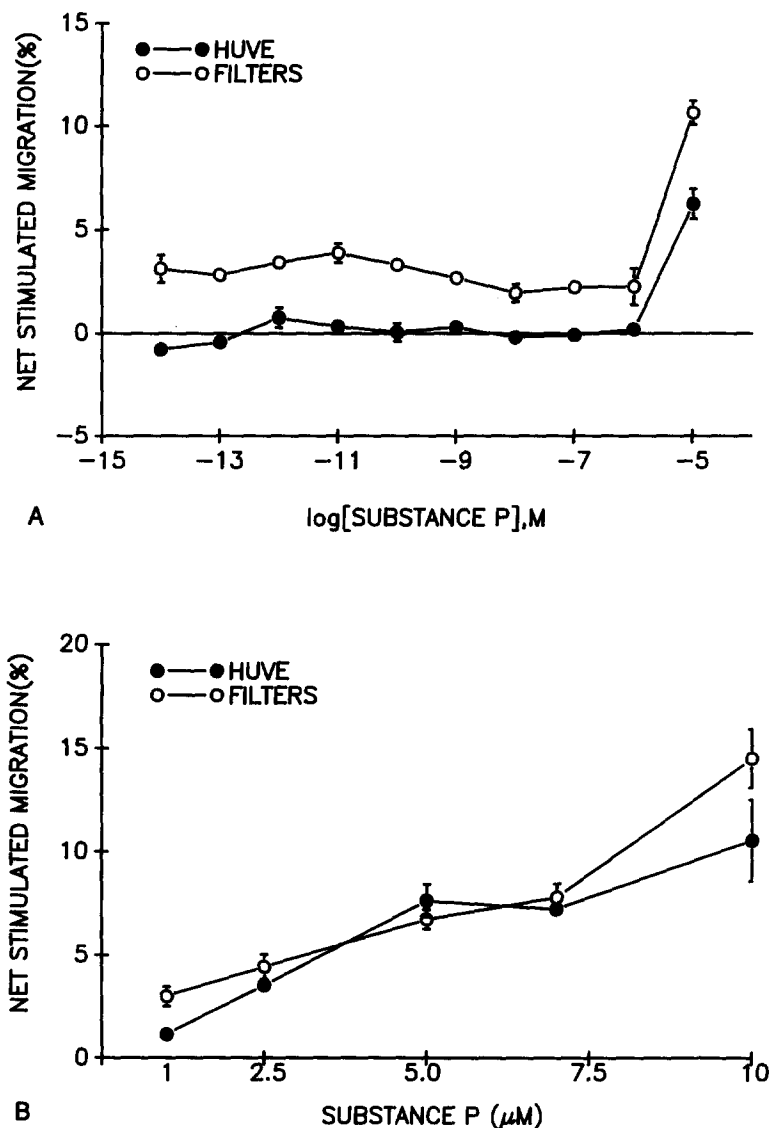


FIG. 1. Dose-response of substance P-induced neutrophil migration across naked filters and HUVE monolayers cultured on filters. Results are expressed as means $\pm$ SEM NSM of ^{51}Cr -labeled neutrophils from nine observations at each dose. **A**, Expanded dose-response experiments; **B**, limited dose-response experiments.

induced neutrophil chemotaxis is shown. Helodermin also did not induce significant neutrophil migration through either barrier at the doses listed.

In the next series of experiments we studied the chemotactic effects of somatostatin and sandostatin. Sandostatin is an octapeptide that has actions similar to those of somatostatin, but it is more resistant to proteases.²⁷ In Fig. 3, *A*, the chemotactic response of neutrophils to somatostatin through HUVE monolayers and filters can be seen. No chemotaxis above background was observed at doses of 10^{-14} to 10^{-5} mol/L. Similarly,

sandostatin had no significant effects on neutrophil migration through either barrier at the doses listed (Fig. 3, *B*).

Because neither VIP nor somatostatin induced significant neutrophil chemotaxis and because both have been reported to inhibit some inflammatory cell functions,^{21, 23, 25} we next examined the effects of VIP and somatostatin on chemoattractant-induced neutrophil migration. VIP and somatostatin at 10 μ mol/L were used to pretreat HUVE cell monolayers and/or neutrophils for 30 minutes. The cells were then washed. Subsequently, we examined the effects of this pretreat-

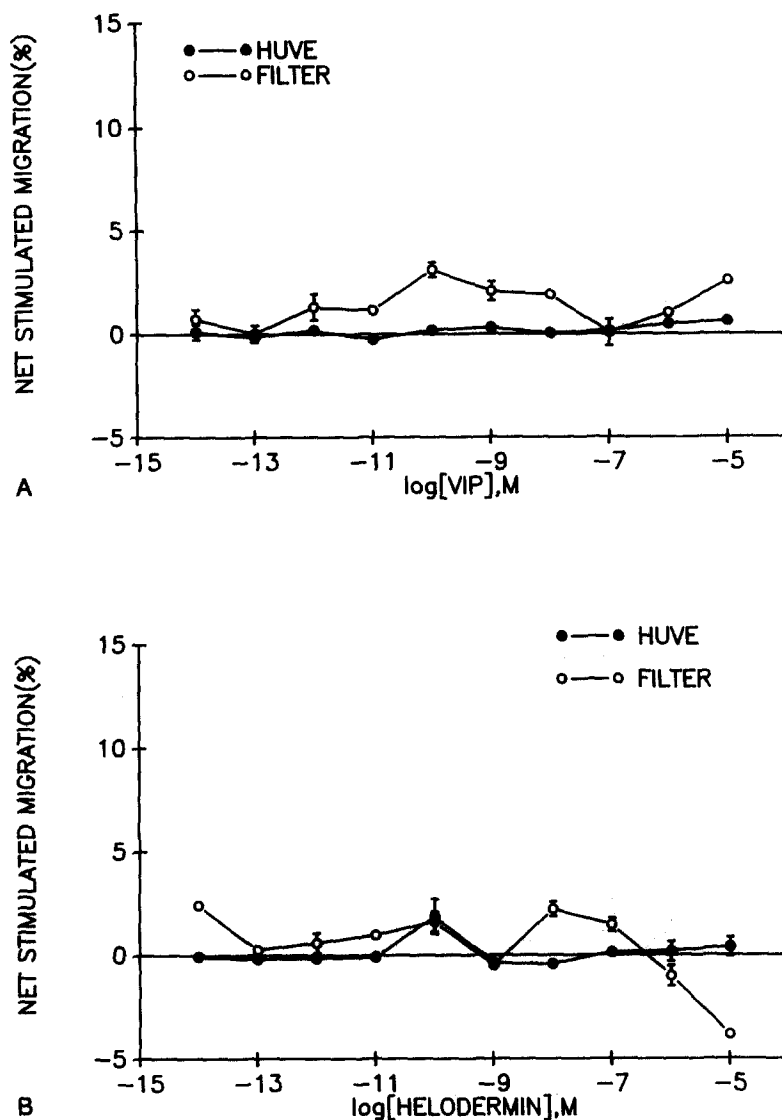


FIG. 2. Dose-response of VIP-induced (A) and helodermin-induced (B) neutrophil migration across naked filters and HUVE monolayers cultured on filters. Results are expressed as means $\pm$ SEM NSM of ^{51}Cr -labeled neutrophils from nine observations at each dose.

ment on neutrophil transendothelial migration in response to FMLP (10^{-10} mol/L), LTB_4 (10^{-9} mol/L), and PAF (10^{-7} mol/L).

Fig. 4, A shows the effects of VIP pretreatment of neutrophils, HUVE cells, and both neutrophils and HUVE cells on FMLP-, LTB_4 -, and PAF-induced transendothelial neutrophil migration. In comparison to percent control migration, measured with no pretreatment, VIP pretreatment regimens did not result in significant inhibition of stimulated neutrophil chemotaxis. VIP pretreatment slightly increased LTB_4 -induced chemotaxis with all three pretreatment regimens. Fig. 4, B shows the effects of somatostatin pretreatment

under identical conditions. Somatostatin pretreatment regimens had no significant effects on FMLP-, LTB_4 -, or PAF-induced transendothelial neutrophil migration when compared with control, untreated values. Although neither VIP nor somatostatin at 10^{-5} mol/L affected neutrophil chemotaxis, we next investigated whether a lower concentration (10^{-10} mol/L) might. We chose 10^{-10} mol/L VIP and somatostatin because this concentration approximated neuropeptide levels measured in animal and human lung tissue.^{32, 33} We again found that neither VIP nor somatostatin affected FMLP-, LTB_4 -, or PAF-induced transendothelial neutrophil migration. The data

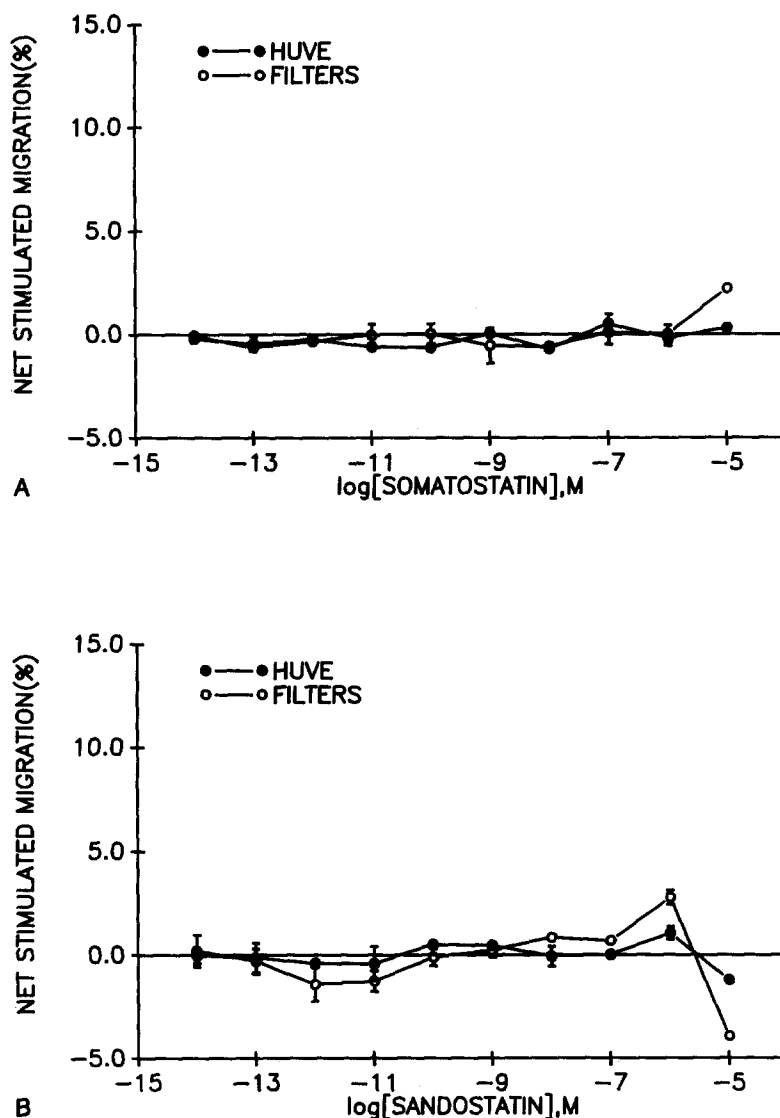


FIG. 3. Dose-response of somatostatin-induced (A) and sandostatin-induced (B) neutrophil migration across naked filters and HUVE monolayers cultured on filters. Results are expressed as means $\pm$ SEM NSM of ^{51}Cr -labeled neutrophils from nine observations at each dose.

from a representative experiment in which PAF was used as the chemoattractant are shown in Fig. 5.

DISCUSSION

There are conflicting data on the chemotactic actions of neuropeptides on human neutrophils, and there are no reports examining the possible interactions among neuropeptides, migrating neutrophils, and the cellular barriers they have to traverse. The object of this study was therefore to examine the effects of neuropeptides on human neutrophil migration through noncellular barriers and through a human endothelial monolayer. We also examined the effects of neuropeptide pre-

treatment of neutrophils and endothelial cells on neutrophil transendothelial migration in response to well-defined chemotactic agents.

We found that substance P is chemotactic for human neutrophils, but only at micromolar doses. Indeed, substance P induced dose-dependent human neutrophil migration through both naked filters and HUVE monolayers at concentrations from 1 to 10 $\mu\text{mol/L}$ (Fig. 1). Moreover, the chemotactic responses through both barriers were relatively equivalent. There are no previous reports on the ability of substance P to induce neutrophil transendothelial migration. However, our data are in agreement with several reports showing that substance P can induce chemotaxis

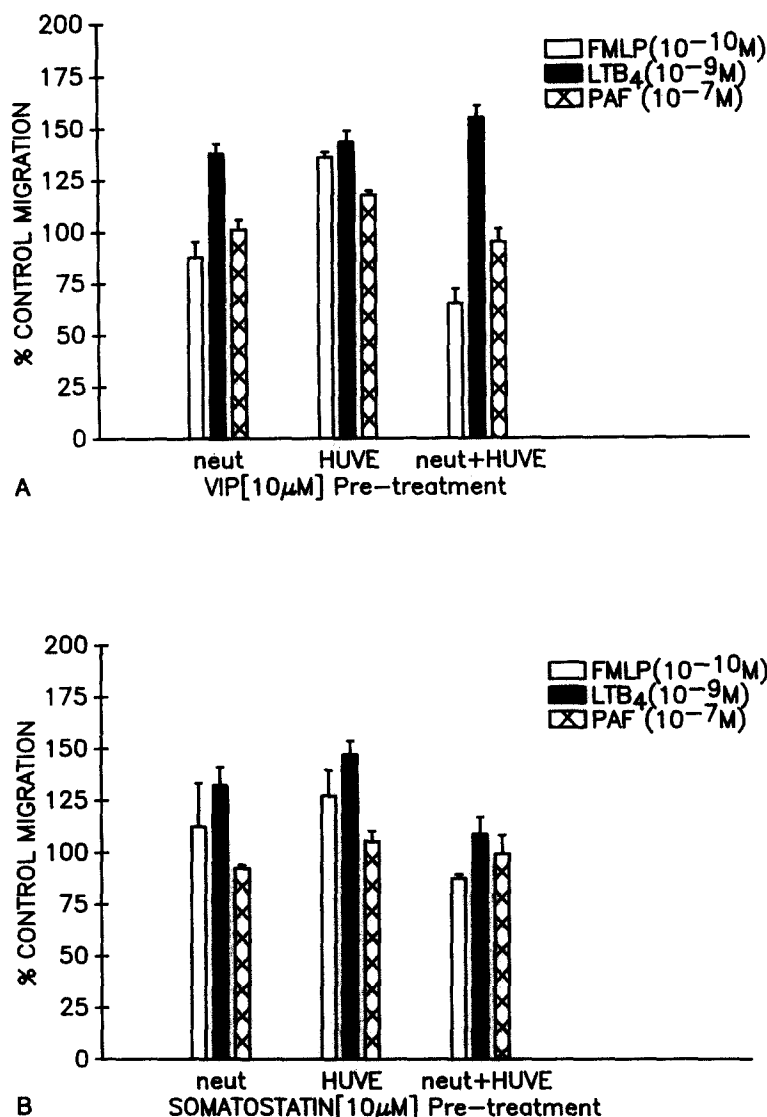


FIG. 4. Effects of VIP (A) and somatostatin (B) pretreatment. The effects of pretreatment of neutrophils and/or HUVE cells by 10^{-5} mol/L VIP (A) or somatostatin (B) on FMLP (10^{-10} mol/L)-, LTB₄ (10^{-9} mol/L)-, and PAF (10^{-7} mol/L)-induced ⁵¹Cr-labeled neutrophil migration through HUVE monolayers cultured on filters are shown. Results are expressed as means $\pm$ SEM of percentage control migration from nine observations of each treatment. Control NSM (i.e., FMLP, LTB₄, or PAF alone) was generally greater than 20%.

of rabbit,¹⁵ rat,¹⁶ and human¹⁹ neutrophils through noncellular barriers at micromolar concentrations. In contrast, Weidermann et al.¹⁸ found that substance P induced predominantly chemokinetic movement of neutrophils and that this effect occurred at concentrations of 1 to 100 nmol/L. Overall, the majority of data suggest that substance P does have chemotactic capacity, albeit at rather high doses.

Neither VIP, somatostatin, nor their relatively protease-resistant analogs, helodermin and sandostatin, exhibited any chemotactic activity at con-

centrations from 10^{-14} to 10^{-5} mol/L through either naked filters or HUVE monolayers. In contrast, Rabier et al.¹⁹ found that VIP and a somatostatin analog (BIM 23014) induced neutrophil chemotaxis through polycarbonate filters. Using a sealed capillary migration test, Bondesson et al.¹⁷ found that VIP inhibited human neutrophil and monocyte chemotaxis at doses less than 1 nmol/L but stimulated neutrophil and monocyte migration at doses of 10^{-13} and 10^{-14} mol/L. They also reported that helodermin had no effect on either neutrophil or monocyte chemotaxis at any

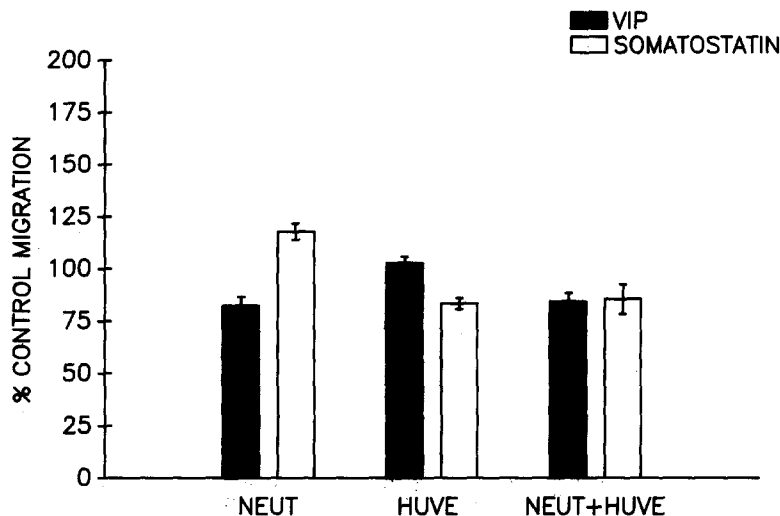


FIG. 5. Effects of VIP and somatostatin pretreatment on PAF-induced transendothelial migration of neutrophils. The effects of pretreatment of neutrophils and/or HUVE cells by 10^{-10} mol/L VIP or somatostatin on PAF (10^{-8} mol/L)-induced ^{51}Cr -labeled neutrophil migration through HUVE monolayers cultured on filters are shown. Results are expressed as means $\pm$ SEM of percentage control migration from three observations of each treatment. Control NSM (i.e., PAF alone) was 27%.

dose.¹⁷ Thus the published data concerning the neutrophil chemotactic capacity of VIP, somatostatin, helodermin, and somatostatin analog are discordant. Despite use of a wide range of concentrations, we were unable to show that these agents had any direct effects on neutrophil chemotaxis through either naked filter or endothelial cell barriers (Figs. 2 and 3). Thus our data suggest that it is unlikely that the small effects noted by previous investigators are of physiologic significance, given the lack of positive effects of these agents in inducing neutrophil migration through more physiologically relevant endothelial barriers.

Although VIP and somatostatin did not directly affect neutrophil migration, we postulated that they might significantly inhibit neutrophil chemotaxis induced by other stimuli. This hypothesis was based on numerous studies showing that both VIP and somatostatin exhibit antiinflammatory actions. For example, VIP and somatostatin have been found to inhibit cytokine production by mononuclear cells²³ and to inhibit lymphocyte proliferation.^{21, 25} Moreover, VIP has been shown to inhibit lung injury induced by hydrochloric acid and paraquat,²⁴ two stimuli that might in part induce lung injury via neutrophil-dependent mechanisms. We therefore examined the effects of VIP and somatostatin on neutrophil transendothelial migration in response to three chemoattractants, FMLP, LTB₄, and PAF. Despite pre-

treatment of neutrophils, endothelial cells, or both neutrophils and endothelial cells with VIP and somatostatin, no inhibitory effects on neutrophil chemotactic responses were observed (Figs. 4 and 5). In fact, VIP pretreatment slightly enhanced LTB₄-induced transendothelial neutrophil migration. Therefore we conclude that VIP and somatostatin, although shown to have antiinflammatory effects, do not significantly inhibit neutrophil chemotaxis.

The direct chemotactic effects of neuropeptides on neutrophils, which are important immune effector cells in the lung, appear small. Indeed, only substance P induced neutrophil transendothelial migration, and this effect was only noted at high doses. Whether or not the *in vitro* chemotactic capacity of substance P demonstrated in this study correlates to a possibly similar *in vivo* effect is not known. Direct measurement of substance P in tissues is difficult because of the rapid degradation of this peptide. Levels of substance P in animal and human lung samples are in the picomolar to nanomolar range.^{32, 33} However, it is unclear whether local concentrations released near nerve terminals are greater. Indeed, substance P has been shown to induce the expression of an endothelial-leukocyte adhesion molecule by microvascular endothelium.³⁴ Moreover, intravenously administered capsaicin, a compound known to release substance P from sensory nerves, has been shown to induce neutrophil ad-

herence to vascular endothelium of rat trachea.¹⁰ These data suggest that concentrations of substance P that directly affect neutrophil adherence, and possibly chemotaxis, are achievable *in vivo*.

Despite the paucity of data indicating a substantive role for these neuropeptides in directly modulating neutrophil chemotactic responses, there are significant data to support their importance in inflammatory responses in the respiratory tract. Stimulation of sensory nerves has been shown to induce mucosal permeability mediated largely through substance P, and this effect can be inhibited by somatostatin.^{4, 11} Furthermore, the neuropeptides studied have been shown to directly affect the release of cytokines,^{12, 20, 22} which in turn can modulate neutrophil and other inflammatory cell functions. For example, substance P has been reported to upregulate the expression of the neutrophil chemotactic factor, interleukin-8, from airway cells¹² and to induce the production of other cytokines from mononuclear cells.²² Therefore although the neuropeptides studied did not have profound direct effects on neutrophil chemotaxis, they are likely to be important modulators of neutrophil-rich inflammatory cell responses through their effects on other cells. This may include the generation of cytokines with neutrophil-activating and chemotactic capacity, as well as the expression of endothelial cell adhesion molecules.³⁴

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Eosinophil major basic protein enhances the expression of neutrophil CR3 and p150,95

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Background: We examined the effect of major basic protein (MBP) stimulation on the expression of the neutrophil β_2 -integrins: LFA-1 (CD11a/CD18), CR3 (CD11b/CD18), and p150,95 (CD11c/CD18).

Methods: Incubation of neutrophils with 0.75 to 3.0 $\mu\text{mol/L}$ MBP for 30 minutes at 37°C resulted in concentration-dependent increases in CR3 expression as detected by staining with the CD11b-specific monoclonal antibody, Leu-15.

Results: The expression of CR3 was significantly ($p < 0.001$) higher when neutrophils were stimulated with 1.5 $\mu\text{mol/L}$ (53% $\pm$ 9% increase) or 3.0 $\mu\text{mol/L}$ (100% $\pm$ 17% increase) MBP as compared with unstimulated neutrophils. The kinetics of MBP-stimulated CR3 expression were rapid and were similar to those of 100 nmol/L

N-formyl-methionyl-leucyl-phenylalanine-stimulated enhancement of CR3 expression.

Incubation of neutrophils with reduced and alkylated MBP resulted in significantly lower ($p < 0.05$) increases in CR3 expression as compared with stimulation with native MBP. In addition, neither eosinophil cationic protein nor eosinophil-derived neurotoxin altered neutrophil CR3 expression. MBP stimulated minimal increases in LFA-1 expression. However, staining with the monoclonal antibody Leu-M5 (anti-CD11c) revealed that MBP stimulated significant increases in the expression of p150,95.

Conclusions: These results indicate that MBP stimulates the increased expression of neutrophil adhesion molecules, which in turn may enhance the inflammatory role of neutrophils in the late-phase events of allergic diseases. (*J ALLERGY CLIN IMMUNOL* 1993;92:598-606.)

Key words: Major basic protein, eosinophils, neutrophils, integrins, CR3, p150,95, adhesion

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