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A soluble gradient of the neuropeptide secretoneurin promotes the transendothelial migration of monocytes in vitro

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Abstract

Secretoneurin, derived from the chromogranin secretogranin II, triggers the selective migration of human monocytes, eosinophils, fibroblasts, endothelial and smooth muscle cells. More recently, we located specific binding sites on the human monocytic cell line MonoMac-6. Differentiated U937 transendothelial diapedesis was evaluated using an in vitro model of the vascular wall and specific monoclonal antibodies against CD11/CD18 and the α-chains of the very late activation antigen (VLA)-4 were used to evaluate involved adhesion molecules. Results showed a significant migratory response to secretoneurin between 10^{-8} to 10^{-10} M. Migration was comparable to a maximal effect induced by the monocyte chemotactic agent *N*-formyl-Met–Leu–Phe (fMLP, 10^{-8} M). Rabbit anti-secretoneurin antibodies were able to block the neuropeptide effect but not of fMLP and a trypsinized secretoneurin preparation as well as the secretogranin II-fragment EL-17 were ineffective in eliciting migration. Transmigration of U937 across endothelial-layers toward secretoneurin is inhibited by antibodies to CD11/CD18 adhesion molecules. The novel neuropeptide secretoneurin may play a role in regulating migration of monocytes into the subendothelial space, supposing a role in inflammatory responses.

Keywords: U937; Transmigration; Endothelial cell; CD11/CD18; VLA-4 (very late activation antigen)

1. Introduction

Peripheral blood monocytes interact with the vascular endothelial lining as an initial step in a wide range of pathological processes including acute and chronic inflammation, immune reactions and atherosclerosis (Cybulsky and Gimbrone, 1991, 1992; Ross, 1993). In order that the movement of leukocytes from blood into tissues occurs in a site-directed manner, focal factors at the inflammatory lesion determine the capture of circulating leukocytes (Butcher, 1991; Springer, 1994). It was shown that there are at least two general mechanisms by which transendothelial migration may be accomplished. First, the activation of endothelium by proinflammatory cytokines, which cause the endothelium itself to induce transmigration in the absence of an exogenous chemotactic gradient (Moser et al., 1989; Furie and McHugh, 1989; Smith et al., 1991a,b). These stimuli do not induce leukocyte migration directly, but can activate cultured vascular endothelium to induce leukocyte adhesion and transmigration (Issekutz et al., 1995). The second mechanism is the interaction of chemoattractant signals localized to vascular endothelium of the inflamed site, such as interleukin-8 and *N*-formyl-Met–Leu–Phe (fMLP) with their receptors on leukocytes activating several processes, including enhanced adhesive capacity of leukocyte integrin molecules (Diamond et al., 1990; Smith et al., 1991a,b). Once arrested at the endothelial surface, leukocytes may be directed by a chemoattractant gradient to transmigrate between endothelial cells into the perivascular matrix. Thus, the second mechanism is the production of chemotactic factors by inflamed tissue (Goldstein, 1992), which induce migration across vascular endothelium along gradients of soluble chemoattractants (chemotaxis) (Chuluyan et al., 1995). Chemotactic factor-induced migration of monocytes was shown to be indepen-

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dent on other cell types but involves the β2-integrin (CD11/CD18) adhesion molecule complex and in some cases the very late activation antigen 4 (VLA-4), because monoclonal antibodies to these adhesion molecules markedly inhibit migration (Issekutz et al., 1995). However, at the present time, the mechanism of monocyte infiltration into inflammatory sites including regulatory factors are not fully understood and the transmigration step has been the least studied.

Several neuropeptides, including substance P and calcitonin-gene related peptide, may play a role in mediating inflammatory processes such as transendothelial migration of leukocytes. Receptor binding sites for neuropeptides are expressed by arterioles and venules (Hirata et al., 1988; Sanabria and Silva, 1994; Berthiaume et al., 1995). In recent studies, neuropeptides were shown to modulate leukocyte, endothelial and mesenchymal cell functions (Ziche et al., 1991; Kähler et al., 1993a,b; Wiedermann et al., 1993, 1996). Furthermore, substance P stimulates vascular endothelial cells to increase vascular permeability (Iwamoto and Nadel, 1989), promotes leukocyte adherence to endothelial monolayers (Zimmermann et al., 1992), induces transendothelial migration of neutrophils (Nakagawa et al., 1995) and causes granulocytic infiltration in human skin (Smith et al., 1993). Proteolytic processing of secretogranin II leads to the formation of a 33-amino-acid peptide, named secretoneurin, which is co-released with substance P and calcitonin-gene related peptide from afferent C-fibers in the periphery by capsaicin (Kirchmair et al., 1994). Secretoneurin is found in high concentrations in the brain (Vaudry and Colon, 1991; Kirchmair et al., 1993) and was also detected in human plasma (Leitner et al., 1994). Its distribution in tissue is comparable to that of other perivascular neuropeptides (Chronwall et al., 1985; Saria et al., 1994). It seems to act as a proinflammatory neuropeptide regulating migration and proliferation of cells involved in inflammatory responses, as we have demonstrated that secretoneurin acts as a potent modulator of neutrophil, eosinophil, monocyte, fibroblast, smooth muscle and endothelial cell functions in vitro (Reinisch et al., 1993; Kähler et al., 1996, 1997a,b), properties also reported for other neuropeptides (Ruff et al., 1985; Wiedermann et al., 1987). Furthermore, we reported recently that secretoneurin triggers the selective migration of human monocytes in vitro and in vivo (Reinisch et al., 1993) employing a G-protein coupled protein kinase C activation and intracellular Ca^{2+} release (Schratzberger et al., 1996a,b). Additionally, Fischer-Colbrie et al. recently identified high affinity binding sites on human monocytic cell lines (Schneitler et al., 1998).

Thus, in vitro and in vivo observations suggest that secretoneurin might regulate the recruitment of neutrophils and monocytes to inflammatory sites. To investigate this possibility in further detail, we have employed an in vitro transendothelial migration assay that consists of calf pulmonary artery endothelial cells or human umbilical vein endothelial cells, and a polycarbonate membrane bearing 5 μm pores. Differentiated U937 cells, a human monoblast cell line, has been used as a model to study the migratory function of human monocytes. Increasing cyclic AMP (cAMP) enhances the expression of chemotactic receptors independently of U937 cell differentiation (Fischer et al., 1995) and allows directed migration of U937 toward zymosan-activated serum, fMLP and leukotriene B_4 (Gavison et al., 1988). Using this approach, we demonstrate that a soluble gradient of SN leads to a rapid and specific concentration-dependent directed migration of U937 cell line-derived monocytes across a vascular barrier of both arterial and venous origin. Furthermore, we describe for the first time that adhesion/migration mechanisms in monocyte transendothelial migration elicited by secretoneurin is dependent on the β2-integrin (CD11/CD18) but not on VLA-4.

2. Materials and methods

2.1. Secretoneurin and production of antisera

Human secretoneurin (secretogranin II 154–186) was obtained from Neosystem, Strasbourg, France. Bovine secretogranin II 217–286 (EL-17), a trypsinized secretoneurin preparation and antisera were produced as described. For antisera production, secretoneurin was coupled via an additional N-terminal cystein to maleimide-activated keyhole limpet hemocyanin and used for generation of antisera in Chincilla Bastard rabbits (Ivanovas, Kislegg, Germany) as previously reported in detail (Kirchmair et al., 1993). Immunoglobulin fractions were prepared from antiserum by affinity chromatography on Protein A Sepharose columns (Pharmacia, Bromma, Sweden). Proteolytic cleavage of secretoneurin was performed with trypsin (350 μg secretoneurin in 80 μl of 0.1 M Tris–HCl, pH 7.9, containing 10 μg trypsin for 3 h at 37°C). Purity of peptides was verified by amino acid sequencing and mass spectrography and the concentrations were determined by amino acid analysis based on phenylalanine. Contamination by endotoxin was determined by E-TOXATE assay (Limulus amebocyte lysate, Sigma, St. Louis, MO).

2.2. Reagents

fMLP (Sigma) was prepared as a stock solution at 10^{-2} M in phosphate-buffered saline (PBS) and stored at −20°C, and diluted prior to use at 10^{-8} M. Tumor necrosis factor-α (TNF-α, stock solution 10 μg/ml) was obtained from Boehringer-Mannheim (Vienna, Austria) and diluted to final concentrations in assay medium. Dibutyryl cyclic adenosine monophosphate (cAMP) was obtained from Sigma.

2.3. Conditions of cell culture

2.3.1. Human umbilical vein endothelial cells

Briefly, endothelial cells were isolated from umbilical cords after treatment with 0.5 mg/ml collagenase (Schöller Pharma, Vienna, Austria) in 0.01 M PBS as described (Jaffe et al., 1973) and grown in specific medium (Promo Cell, Biomedica, Vienna, Austria) containing 10% fetal calf serum, 0.1 mg/ml epidermal growth factor, 1 ng/ml bovine fibroblast growth factor, 1 μg/ml hydrocortisone. Cells were cultured in gelatin-coated culture flasks and kept in a humidified incubator at 37°C in 5% CO_2. Endothelial cells were used between passages 2 and 4 for all experiments.

2.3.2. Bovine artery endothelial cells

Bovine artery endothelial cells were obtained from the American Type Culture Collection, Rockville, MD. These arterial endothelial cells were previously shown to interact with human polymorphonuclear leukocytes in a manner comparable to human endothelial cells (Strubel et al., 1993). Cells were grown in enriched medium (Medium 199 in Earle's salts base with L-glutamine (Biological Industries, Beth Haemek, Israel), 20% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in 75 cm^2 plastic tissue culture flasks and kept in a humidified incubator at 37°C in 5% CO_2. After reaching confluence the cells were subcultured and reseeded at a ratio of 1:3. Endothelial cells were used between passages 3 and 8 for all experiments.

2.3.3. U937 human monocytic cell line

The U937 monocytic cell line (American Type Culture Collection, Rockville, MD) was maintained in RPMI medium with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin and 10 mM HEPES in a humidified incubator at 37°C in 5% CO_2. The characteristics of the adhesive and transmigratory potency of these cells for cultured vascular endothelial cells have been described previously (Chuluyan and Issekutz, 1993; Ziegelstein et al., 1994). Kew et al. (1997) demonstrated that undifferentiated U937 cells possess the subcellular signaling apparatus and machinery necessary to generate a motile response and that the only missing component for chemotaxis is expression of a chemoattractant receptor. Differentiation of the U937 cell line was induced by addition of dibutyryl cAMP (10^{-3} M) at a cell density of 3×10^5 cells/ml as described previously (Gavison et al., 1988). Studies were carried out on U937 after 48–72 h of exposure to dibutyryl cAMP, since expression of chemotactic receptors like C5a is fully developed at this time (Gavison et al., 1988). The differentiated cells were washed, and resuspended at 1×10^6 cells/ml in RPMI 1640/0.5% bovine serum albumin/10 mM HEPES for migration experiments.

2.4. Nitrocellulose chemotaxis assay

Differentiated U937 cells were screened for their ability of a chemotactic response toward secretoneurin or fMLP, respectively, in a modified multiwell Boyden chamber. To the upper wells of the chemotactic chamber, 5×10^5 monocytes (28 μl) were added and the bottom wells were filled with the chemoattractants or the vehicle control (48 μl). The two wells were separated by a 5 μm pore-sized nitrocellulose filter (Sartorius, Göttingen, Germany) presoaked with chemotaxis medium (RPMI 1640/0.5% bovine serum albumin/10 mM HEPES). Chambers were then incubated for 120 min at 37°C (5% CO_2, fully humidified). Migration depth of the cells into the filter was microscopically measured (leading-front assay) in quadriplicate, after the cells and filters were fixed, dehydrated and stained with hematoxylin.

2.5. Transendothelial chemotaxis assay

The migration assay was performed as described previously (Kitayama et al., 1997) using modified Boyden chambers with a 6.5 mm diameter, 5 μm pore size, 10 μm thickness polystyrene membrane separating the two chambers (Transwell, Costar, NL) with some modifications. Endothelial cells were seeded on polystyrene filters of transwell culture plate inserts coated with 0.01% gelatine (37°C, 18 h) followed by application of 60 μg/ml of human fibronectin (Collaborative Research, Meylan Cedex, France) at 37°C for 2 h. One week after seeding, endothelial cells formed a tight permeability barrier and each batch was tested for confluence by microscopic control and fluorometric evaluation for endothelial barrier function as described below. Medium was exchanged for fresh medium two days before use on day 6 or 7. In experiments employing activated endothelial cells, monolayers were prestimulated with TNF-α (10 ng/ml) for 5 h. After this, the upper and lower compartments were washed twice with Hank's balanced salt solution (HBSS) and transferred to a new clean lower compartment which was filled with either medium (control) or test chemoattractant substances dissolved in 0.6 ml RPMI 1640/0.5% bovine serum albumin/10 mM HEPES. Thereafter, 1×10^5 differentiated monocytic U937 cells in 100 μl medium were added to the upper compartment. For selected experiments, rabbit anti-secretoneurin antibodies were added to the lower compartments at a final dilution of 1:1000. After incubation for the indicated time points at 37°C, migration was stopped by gently washing of the upper compartment twice with 0.1 ml of HEPES/0.5% EDTA to remove nonadherent monocytes. As described previously, transmigration of monocytes reaches a maximum after 3 to 4 h of incubation, at which time approximately 40% of monocytes have passed the barrier (Hakkert et al., 1990). The transwells were then incubated for 30 min at 4°C and membrane-bound mono-

cytes collected into the lower compartment by centrifugation at 1500 rpm for 5 min. Monocytes in the lower compartment were resuspended and allowed to settle for 30 min. Cells at the bottom of the lower compartment were finally evaluated by cell counting in three microscopic fields/cup at a 100-fold magnification using an inverted field microscope. Results are partially given as transmigration index, which is the number of cells per microscopic field that have transmigrated toward test substances, divided by the number of cells per microscopic field that migrated toward medium. All experiments were performed in duplicate.

2.6. Monoclonal antibody treatments

In some selected experiments, differentiated U937 monocytic cells were treated for 30 min at room temperature with purified monoclonal antibodies TS 1/18 against β2-integrin (CD11/CD18) adhesion molecule complex or against the α-chain of VLA-4 (Endogen, Woburn, MA) at saturating concentrations (20–40 μg/ml; Chuluyan and Issekutz, 1993), and then tested for migration in the presence of the antibodies throughout the transmigration experiment.

2.7. Test of endothelial monolayer permeability

Endothelial cells grown on gelatine/fibronectin-coated polycarbonate filters form a tight permeability barrier after 5 to 7 days of incubation as described previously (Hakkert et al., 1990). To evaluate the functional integrity of the endothelial cell monolayers, they were tested for their permeability to fluorescein isothiocyanate conjugate (FITC)-labelled bovine serum albumin (Sigma). Cells were cultured on Transwell inserts as described above. For measuring permeability, 100 μl of FITC-labelled bovine serum albumin (100 μg/ml) was added to the upper compartment after removal of medium and gently washing with HBSS. The Transwells were then placed into new clean 24-well trays, the lower compartment containing 600 μl of HBSS. After 60–180 min of incubation at 37°C, 5% CO_2, the transwells were removed, 50 μl were sampled from the lower and upper compartment and fluorescence measured using the Cytofluor 2350 measurement system (Millipore, Bedford, MA). Transwells without endothelium (pretreated filters alone) were used for comparison. Diffusion of bovine serum albumin was expressed as a percentage of equilibrium. In the absence of an endothelial cell monolayer on the polycarbonate membrane, FITC–bovine serum albumin equilibrium was up 70% within minutes. Bovine serum albumin diffusion was generally less than 5% of added fluorescence when monolayers were grown to confluency. Endothelial monolayers which showed permeability over 5% were discarded.

2.8. Statistical analysis

All migration experiments were performed in duplicate and repeated three to more times. P values were calculated by Student's t-test or Mann–Whitney U-test (StatView, Abacus Concepts, Berkeley, CA) and the differences with $P < 0.05$ were considered to be significant.

3. Results

3.1. Secretoneurin-dependent U937-derived monocyte migration into nitrocellulose filters

It was recently shown that secretoneurin elicits directed migration of peripheral mononuclear cells in vitro and in vivo (Reinisch et al., 1993). Thus, in order to test the ability of differentiated U937 to respond chemotactically toward this novel neuropeptide, we first evaluated chemotaxis employing a multiwell Boyden chamber and nitrocellulose micropore filters. As depicted in Fig. 1, secretoneurin elicited a dose-dependent bell-shaped migration of U937 cells at concentrations of 10^{-5} M to 10^{-10} M with a maximum at 10^{-8} M after 120 min of incubation. fMLP served as a positive chemoattractant control at a concentration of 10^{-8} M. The migratory response evoked by secretoneurin was nearly comparable to that elicited toward the well characterised monocyte chemotactic peptide fMLP. No significant migratory response was observed toward the neuropeptide secretoneurin at concentrations tested less than 10^{-10} M.

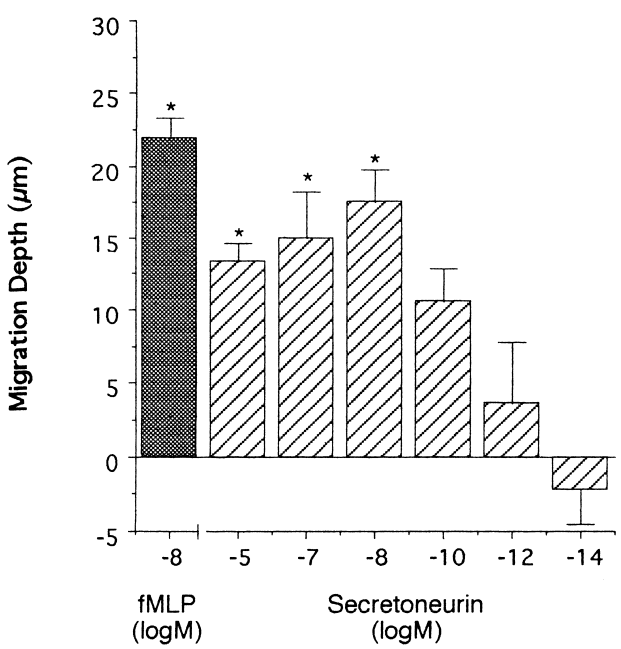

Fig. 1. U937 cell migration into a nitrocellulose membrane induced by secretoneurin. Dose response curve is shown at log M dilutions. U937 (1×10^6 cells/ml) were added to the upper compartment as described in Section 2. Data are means ± S.E.M. of the difference between the distance of migration toward test substances and that toward buffer alone after 120 min; $n = 8$. * $P < 0.05$ (Mann–Withney U-test).

3.2. Secretoneurin-dependent transendothelial migration

The optimal concentration tested for differentiated U937 monocytic cell diapedesis across endothelial monolayers toward secretoneurin was determined in dose–response experiments over a 240-min incubation period (optimal time period). Consequently, we first tested the ability of differentiated U937 to migrate in response to the neuropeptide over a broad concentration range (from 10^{-6} M to 10^{-14} M). Results showed a significant migratory response of U937 monocytic cells through a human umbilical vein endothelial cell monolayer toward secretoneurin (Fig. 2a). The observed migratory effect was concentration-dependent, showing a bell-shaped curve with a peak response occurring at 10^{-8} M. The peak migratory effect on monocytic U937 cells toward secretoneurin was comparable to the chemotactic effect seen in the control group using fMLP (10^{-8} M) as transendothelial chemoattractant. Endotoxin contamination of peptides was excluded by a sensitive Limulus amebocyte lysate assay detecting reactivity neither in the secretoneurin nor in the fMLP preparations. To estimate whether the observed effect is peculiar to a certain endothelial cell barrier, we investigated the effect on monocytic U937 cell transmigration also through a vascular barrier of arterial origin, employing a calf pulmonary artery endothelial cell monolayer. As described above, secretoneurin also caused a significant increase in U937 monocytic cell transmigration through this arterial monolayer, elucidating a significant transmigratory response between 10^{-7} M to 10^{-10} M, showing a maximal effect at 10^{-10} M (Fig. 2b). In further experiments we determined whether the observed effect is also mediated by another secretogranin II fragment 217–286 unrelated to secretoneurin. For this purpose we tested EL-17 on its transmigratory chemotactic activity through a endothelial barrier at a concentration of 10^{-8} M (Fig. 3). As observed in experiments involving human monocytes for migration through naked filters (Reinisch et al., 1993), EL-17 did not elicit a significant migratory response at the concentration tested (Fig. 4). These observations are consistent with results obtained on chemotaxis through naked filters with peripheral human monocytes, human fibroblasts, endothelial and smooth muscle cells in vitro. Furthermore, secretoneurin unactivated by trypsinization procedure evoked no migratory effect at the optimal concentration tested (10^{-8} M) through endothelial monolayers on human U937 monocytes in our in vitro assay system (Fig. 3).

When TNF-α (10 ng/ml) was used to stimulate monolayers of venous origin in front of chemotactic stimulation there was no significant difference in random U937 monocytic cell transendothelial migration in comparison with migration across unactivated endothelium in our assay

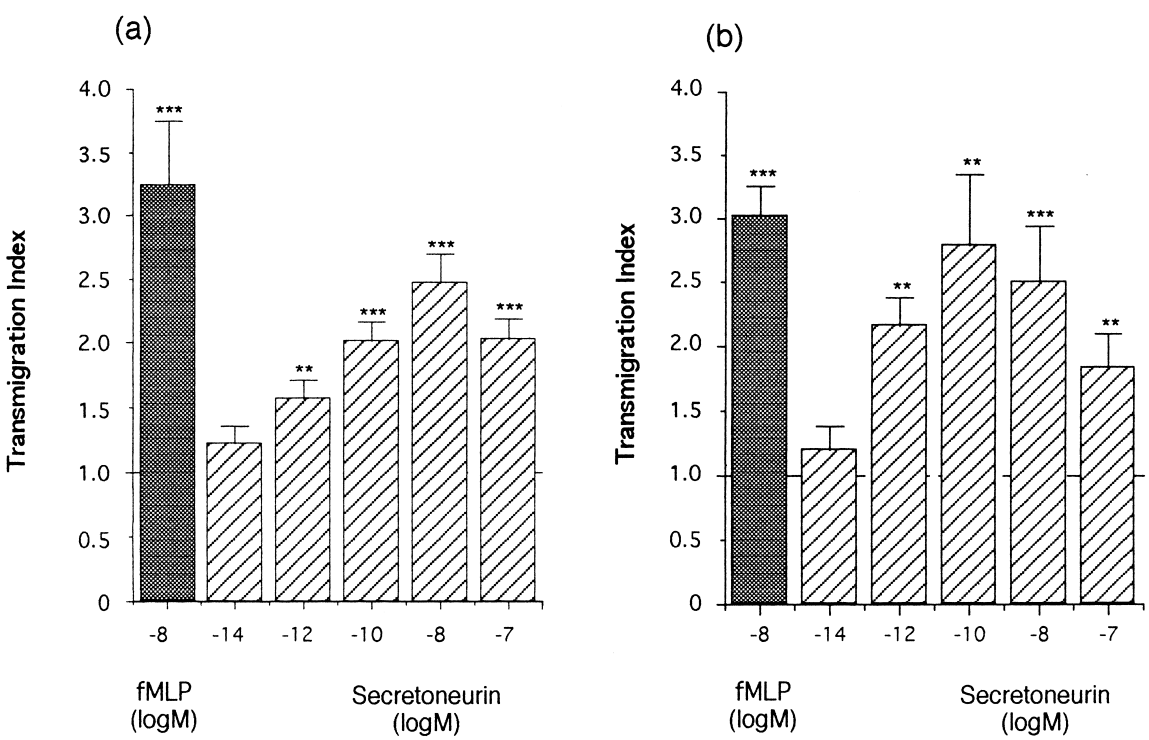

Fig. 2. Dose–response curve of human monocyte transcellular migration through a human umbilical vein (a) and a calf pulmonary artery endothelial cell monolayer induced by secretoneurin. U937 (1×10^5) were added above the endothelial monolayer and stimulated with varying concentrations of secretoneurin added beneath the monolayer/filter unit to induce migration across the endothelial monolayer. The number of monocytes that had migrated across the endothelial barrier and the filter was determined microscopically after 240 min as described under Section 2. Data are means ± S.E.M. of transmigration indices (ratios between number of monocytes migrated vs. test attractant and number of monocytes migrated vs. medium alone; $n = 14$. $**P < 0.01$, $***P < 0.001$ (Student's t-test).

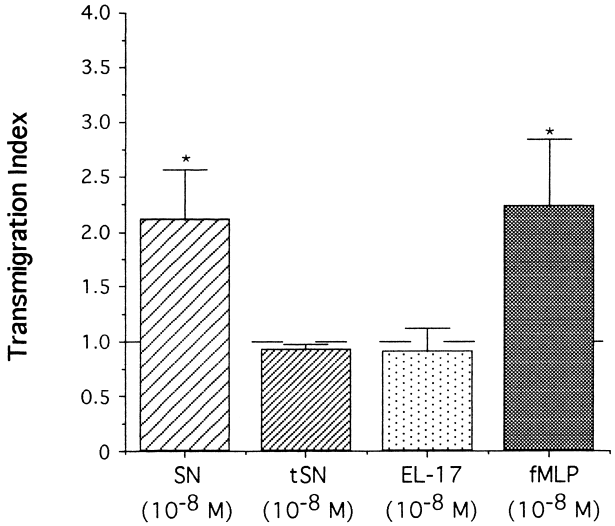

Fig. 3. Effect of a trypsinized secretoneurin preparation and another secretogranin II fragment (EL-17) on human monocyte transendothelial migration. U937 (1×10^5) were added above the calf pulmonary artery endothelial monolayer and stimulated with EL-17 (10^{-10} M), trypsinized secretoneurin (10^{-10} M) or fMLP (10^{-8} M) added beneath the monolayer/filter unit to induce migration across the endothelial monolayer. The number of monocytes that had migrated across the endothelial barrier and the filter was determined microscopically after 240 min. The data shown represent the means ± S.E.M. of transmigration indices (ratios between number of monocytes migrated vs. test attractant and number of monocytes migrated vs. medium alone; $n = 4$. $*$ $P < 0.05$ (Mann–Whitney *U*-test).

system. However, in the presence of TNF-α-activated human umbilical vein endothelial cells, secretoneurin again induced a strong transendothelial migration of human monocytic U937 cells (Fig. 4). The maximal chemotactic response of differentiated U937 cells was not significant different through activated or unactivated confluent endothelial monolayers toward the chemotactic agent secretoneurin or fMLP. Identical results were obtained by Hakkert et al. (1990) on human umbilical vein endothelial cells cultured on collagen matrix, employing interleukin-1 as endothelium stimulatory agent. The permeability of the endothelial monolayer to FITC-labelled bovine serum albumin was not significantly increased by stimulation with TNF-α for 5 h (data not shown).

3.3. Kinetics of secretoneurin-induced U937-derived monocyte transendothelial migration

Time course experiments of secretoneurin-induced monocytic U937 cell transendothelial migration across umbilical vein endothelial cell monolayer barriers were performed using secretoneurin as a chemotactic agent at a concentration of 10^{-8} M (Fig. 5). Migration of differentiated U937 monocytic cells across naked filters began by 2 h and gradually increased reaching a plateau at about 3 h of incubation (data not shown). In contrast, U937 derived monocyte migration across endothelial monolayers toward

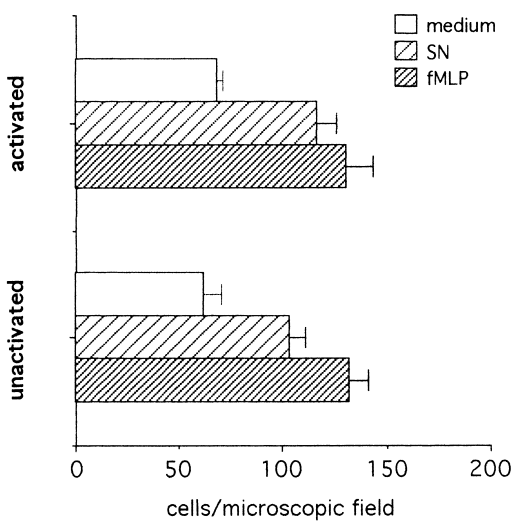

Fig. 4. Human monocyte transendothelial migration through activated and unactivated endothelial monolayers. Human umbilical vein endothelial cells were grown on polycarbonate Transwell inserts and unstimulated or stimulated for 5 h with TNF-α (10 ng/ml). Medium was then exchanged and monocytes added to the monolayers and incubated for 240 min as described in Section 2. Results represent the means ± S.E.M. of counted cells at the bottom of the lower compartment in three microscopic fields/cup at a 100-fold magnification using an inverted field microscope; $n = 5$. $*$ $P < 0.05$ (Mann–Whitney *U*-test).

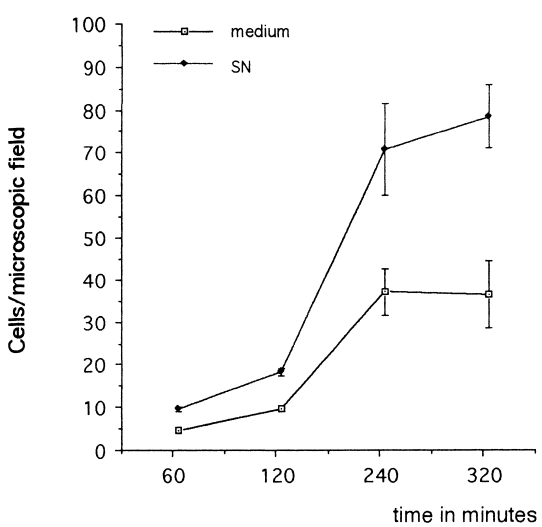

Fig. 5. Time course of human monocyte transendothelial migration induced by secretoneurin. Transendothelial migration was induced with 10^{-10} M secretoneurin or no stimulus as described in Section 2 and stopped after various incubation times. The data shown represent the means ± S.E.M. of counted cells at the bottom of the lower compartment in three microscopic fields/cup at a 100-fold magnification using an inverted field microscope. Each point represents the mean value for duplicate wells; $n = 5$. $*$ $P < 0.05$ (Mann–Whitney *U*-test).

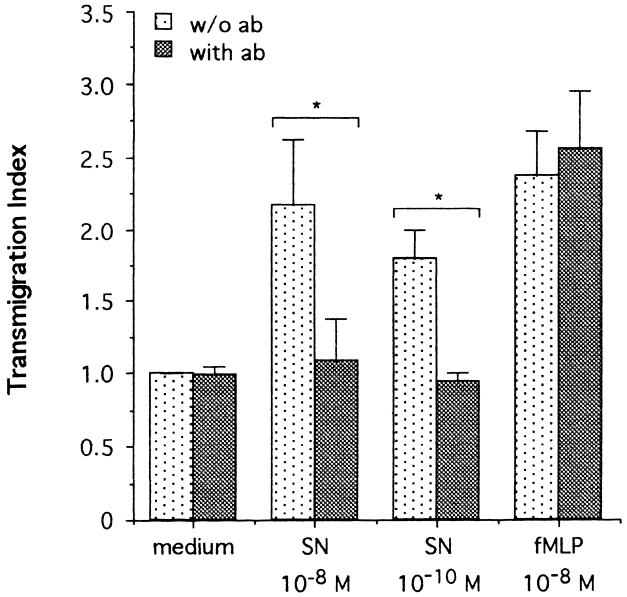

Fig. 6. Antagonising effect of specific anti-secretoneurin antibodies on transmigration of human monocytes toward secretoneurin. U937 (1×10^5) were added above the calf pulmonary artery endothelial cell monolayer and stimulated with secretoneurin (10^{-10} M) or fMLP (10^{-8} M) added beneath the monolayer/filter unit to induce migration across the arterial endothelial monolayer. For selected experiments a dilution of 1:1000 of specific anti-SN antibodies were added to the lower compartment. The number of monocytes that had migrated across the endothelial barrier and the filter was determined microscopically after 240 min. The data shown represent the means ± S.E.M. of transmigration indices (ratios between number of monocytes migrated vs. test attractant and number of monocytes migrated vs. medium alone; $n=6$. $*$ $P<0.05$ (Mann–Whitney *U*-test).

the neuropeptide (10^{-8} M) did not occur to a significant extent over the first hour. However, a sharp increase in transendothelial migration was observed over the subsequent time period, as already reported by Randolph and Furie (1995) (Fig. 5), reaching a plateau after 4 to 5 h of incubation.

3.4. Neutralizing antibody

In order to confirm that the observed transmigratory response of human U937 monocytic cells is due to a specific effect of the investigated neuropeptide, we evaluated the cellular response in the presence of specific anti-secretoneurin antibodies. The anti-secretoneurin-serum was fully effective in inhibiting U937-derived monocyte transmigration, when anti-secretoneurin antibodies were added to the lower compartment at a dilution of 1:1000 in the presence of a chemotactic gradient of the neuropeptide, while antibodies alone did not alter recruitment of U937 monocytic cells into the subendothelial space (Fig. 6). To further examine the specificity of the inhibitory effects of anti-secretoneurin antibodies, we performed further experiments. Thus, we examined whether anti-secretoneurin immunoglobulins could inhibit the chemotactic effects of an irrelevant chemoattractant, fMLP, through an endothelial monolayer. In the case of fMLP (10^{-8} M), monocyte recruitment was not inhibited by the anti-secretoneurin antibodies. These results confirm the specificity of both the neuropeptide-induced chemotactic responses and the inhibitory effects of anti-secretoneurin antibodies on these observed migratory response through endothelium in vitro.

3.5. Requirement for CD11/CD18 but not CD49d in secretoneurin-dependent U937-derived monocyte migration

The adhesion molecules involved in secretoneurin-dependent transendothelial migration across unactivated human umbilical vein endothelial cells were studied by using blocking monoclonal antibodies against molecules on the surface of the monocyte and endothelial cells. Fig. 7 shows that monoclonal antibody preparation TS 1/18 against the common CD11/CD18 β_2 integrin chain (Endogen, Woburn, MA), but not control antibody against CD14 (Serotec, Oxford, UK) inhibited secretoneurin-induced U937-derived monocyte transendothelial migration to a significant extent. As transmigration was inhibited not completely (~ 81.5%), these data suggest the presence of a CD11/CD18-independent mechanism involved in monocyte secretoneurin-dependent transendothelial migration.

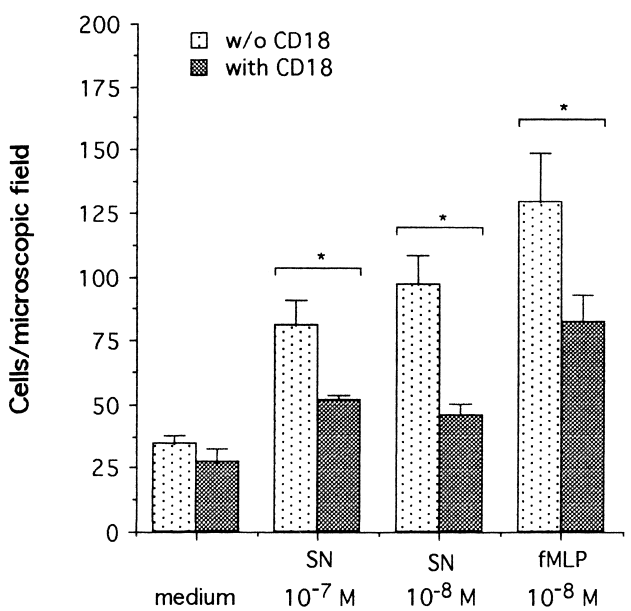

Fig. 7. Effect of monoclonal antibodies against CD18 on secretoneurin and fMLP-induced monocyte transmigration. U937 (1×10^5 cells) treated with control mAb or treated with mAb against CD18 were tested for secretoneurin or fMLP-induced transendothelial migration through unactivated human umbilical vein endothelial cells. The number of monocytes that had migrated across the endothelial barrier and the filter was determined microscopically after 240 min. The data shown represent the means ± S.E.M. of counted cells at the bottom of the lower compartment in three microscopic fields/cup at a 100-fold magnification using an inverted field microscope; $n=6$. $*$ $P<0.05$ (Mann–Whitney *U*-test).

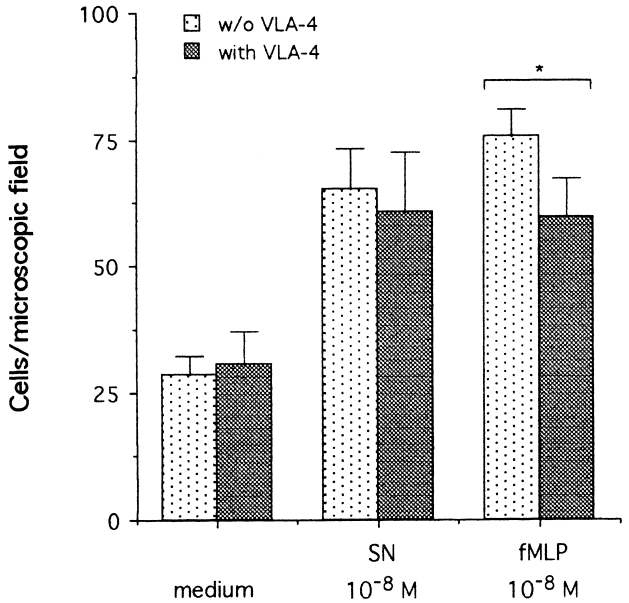

Fig. 8. Effect of mAb against VLA-4 on secretoneurin and fMLP-induced monocyte transmigration. U937 (1×10^5 cells) treated with control with mAb against VLA-4 were tested for secretoneurin or fMLP-induced transendothelial migration through unactivated human umbilical vein endothelial cells. The number of monocytes that had migrated across the endothelial barrier and the filter was determined microscopically after 240 min. The data shown represent the means ± S.E.M. of counted cells at the bottom of the lower compartment in three microscopic fields/cup at a 100-fold magnification using an inverted field microscope; $n=6$. $*$ $P<0.05$ (Mann–Whitney U-test).

As reported previously, VLA-4 participates in chemotactic monocyte transmigration (Issekutz et al., 1995) toward several chemotactic agents like C5a. VLA-4 participation in CD11/CD18-independent migration on unactivated human umbilical vein endothelial cells was confirmed by preincubating the cells with the blocking monoclonal antibodies HP2/1 (Fig. 8). Blocking monoclonal antibodies against the α_4-chain of VLA-4 did not inhibit significantly the secretoneurin-induced migration across unactivated endothelial cells, even there was a tendency for an inhibitory effect. However, data suggest that VLA-4 is not involved in this migratory process elicited by secretoneurin. In contrast, fMLP (10^{-8} M)-induced transmigration was altered to a significant extent, as already reported (Issekutz et al., 1995).

4. Discussion

Neuropeptides have been implicated in the modulation of a variety of cellular responses in neurogenic inflammation and wound healing (Skerret, 1990; Payan, 1992; Wiedermann et al., 1994). Furthermore, the novel neuropeptide secretoneurin was recently shown to evoke potent regulatory cellular responses, e.g., chemotactic responses of human monocytes and eosinophils through naked filters (Reinisch et al., 1993; Dunzendorfer et al., 1998). As secretoneurin is released from peripheral nerve endings and therefore can be found in the substratum to a higher amount as in the circulating blood stream (Leitner et al., 1994), we were interested in the role played by secretoneurin in monocyte transmigration of endothelial monolayers using an in vitro system imitating the arterial or venous vessel wall.

Currently, at least two general mechanisms are recognized to be involved in leukocyte emigration into inflammatory sites. In vivo, endothelial and other inflammatory cells, like mast cells, promote extravasation by participating in adhesive interactions with leukocytes or by secreting chemotactic cytokines, called chemokines, that may facilitate the migration of leukocytes into the underlying substratum. Chemoattractants have been shown to play a critical role by activating integrin adhesiveness and inducing directional movement across the endothelial layer and are thought to guide subsequent migration within tissues toward the inflammatory site. The results reported here demonstrate that human monocytes can transmigrate across an endothelial monolayer by using the novel neuropeptide as a chemotactic factor. Our results show that secretoneurin is able to stimulate U937-derived monocyte transcellular migration through both arterial and venous endothelial monolayers cultured on polycarbonate filters in vitro. Both barriers, are relevant to in vivo inflammatory responses. Human umbilical vein endothelial cells were chosen because monocyte migration from the vasculature is known to occur at the level of postcapillary venules. Moreover, these cells have been shown to express adhesion molecules to be important in monocyte chemotactic responses. In order to suggest a potential generalisability of our results on venous endothelial cells on monocyte transmigration into the subendothelial space, we choose an arterial cell line (calf pulmonary artery endothelial cells), with regard to atherogenic processes, which was shown to interact with leukocytes in a pathophysiological manner (Takahashi et al., 1994). Both monolayers formed a tight permeability barrier to monocyte extravasation in our standardized in vitro model of transendothelial migration. In the absence of a chemotactic gradient (e.g., fMLP), U937-monocyte migration through endothelial monolayers from both origin was usually less than a third of transmigration indices versus a chemotactic gradient of fMLP and consistently less than one half of that through naked filters after 4 h. Thus, our endothelial monolayers are intact, confluent, and impose a true physiological barrier to the transmigration of leukocytes in the absence of a chemotactic gradient as already described by other groups. As peripheral blood monocyte migration and adherence is influenced by the isolation and purification procedure, we examined the effect on secretoneurin-induced transmigration on a homologous monocytic cell population deriving from U937 cells, which is a monocyte-like cell line. In studies done by other groups they showed a variance of human peripheral monocytes transmigrated as proportion of cells among mono-

cytes added considerably from 30 to nearly 100% (Takahashi et al., 1994). After treatment of the U937 cells with dibutyryl cyclic AMP for 72 h, they acquire the capacity to migrate in response to chemotactic agents comparable to human mature monocytes (Gavison et al., 1988). Our in vitro study showed rapid transmigration of monocytes toward secretoneurin located in the lower chamber. The kinetic of monocyte transmigration reaching a plateau after 4 h of incubation is similar to observations by Takahashi et al. (1994). In contrast, monocyte chemotaxis through naked filters plateaus at a lower level by 90 to 120 min. Dose–response data showed a significantly increased differentiated U937-derived monocyte migration through both types of endothelial monolayers (arterial and venous origin) over a wide concentration range (10^{-7} M to 10^{-12} M). In additional experiments, U937-derived monocyte migration through naked nitrocellulose micropore filters was evaluated and monocyte chemotactic response reached a maximum at 10^{-7} M to 10^{-8} M, when secretoneurin was present in the lower compartment. Differences in several methodical aspects may be responsible for the different potency of secretoneurin effects in transendothelial migration assays as described above. Under physiological conditions peripheral blood monocytes adhere to vascular endothelium and leave the circulation within 24 to 48 h (Chuluyan et al., 1995). This emigration is dramatically increased at sites of inflammation and injury. However, in vitro, monocyte transendothelial migration appears much more slower than migration employing naked filter chemotaxis assays. As a consequence, we observed a significant monocyte migration in both tested assays (Boyden Chamber, Transwell) with different time kinetics and even with a slight shift in the maximal dose response. The differences in kinetics between transendothelial migration and migration through naked filters may be explained by a retarded diffusion of chemoattractants (about 8–10 times slower; Kitayama et al., 1997) and the possible interaction with other inhibitory substances or other desensitizing chemoattractants released by the endothelium. Another possibility is the interaction of secretoneurin with the endothelial monolayer causing expression of adhesion molecules, e.g., platelet–endothelial cell adhesion molecule-1 (PECAM-1), on endothelial cells, besides its chemotactic potency resulting in modification of monocyte transmigration.

To further explore whether the observed migratory response is due to a specific effect of secretoneurin, we investigated the effect of the neuropeptide in the presence of specific anti-secretoneurin antibodies. The migratory response of human U937-derived monocytes toward the neuropeptide was abolished in the presence of these specific antibodies, indicating that the observed stimulation is due to a specific effect of this novel neuropeptide. These results are consistent with previous observations obtained with fibroblasts, endothelial and smooth muscle cells when the same antibody preparation was used (Reinisch et al., 1993; Kähler et al., 1996, 1997a,b,c). Additionally, an unactivated, trypsinized secretoneurin-preparation and EL-17, another secretogranin II derived peptide were ineffective in eliciting a transmigratory response. This data demonstrate that secretoneurin itself was, indeed, responsible for the observed effect.

During the inflammatory response, specific cell adhesion molecules on the surfaces of the leukocytes and endothelial cells mediate the initial loose contact and tight adhesion of leukocytes to the apical surface of the endothelium. During the subsequent process of transmigration, tight leukocyte–endothelial contacts are seen in vivo and in vitro. Under most conditions in vitro and in vivo, the leukocyte CD11/CD18 or β2 integrin complex is required for polymorphonuclear leukocyte and monocyte migration (Furie et al., 1991; Chuluyan and Issekutz, 1995) However, the role of CD11/CD18 integrins in chemotactic-factor-dependent migration of monocytes is only partial because monoclonal antibodies against the common subunit (CD18) was shown to only partially (about 75%) inhibit transmigration toward classical chemoattractants (Issekutz et al., 1995). As shown in Fig. 7, secretoneurin-induced transmigration across unstimulated human umbilical vein endothelial cells was inhibited by monoclonal antibodies against CD11/CD18 up to 81%, suggesting involvement of this adhesion molecule complex also in secretoneurin-induced differentiated U937-derived monocyte migration. However, total blockade of monocyte transmigration was not obtained as already described for other chemoattractants. Besides leukocyte adhesion molecules endothelial cell surface molecules, i.e., E-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1) are involved in the transmigratory process. These molecules on the endothelium interact with sialyl Lewis X carbohydrate-containing molecules, CD11/CD18, and VLA-4, respectively on leukocytes (Springer, 1994). Recently, Chuluyan et al. (1995) demonstrated that CD18-independent transmigration in response to classical chemoattractants, i.e., fMLP, is mediated in part by the VLA-4 integrin. In contrast, to observations on fMLP and C5a, secretoneurin-induced transendothelial migration was not inhibited by specific monoclonal antibodies against VLA-4 (Fig. 8). These data suggest the involvement of another pathway for monocyte transmigration toward a secretoneurin gradient. Besides activation of monocytes also an activation of endothelial cells by secretoneurin could take place. Thus, it was recently shown that secretoneurin affects endothelial cell functions and causes an increase in intracellular calcium flux in human umbilical vein endothelial cells (Kähler et al., 1997a). Furthermore, we demonstrated that activation of human umbilical endothelial cells leads to the enhanced expression of PECAM-1 (Kähler et al., 1997a), which was recently shown to be critical in monocyte transmigration (Muller et al., 1993; Rattan et al., 1996). Taken together, our results suggest that a dynamic interaction of monocyte, barrier

cells and secretoneurin leads to enhanced transendothelial migration of human differentiated U937-derived monocytes in a dose- and time-dependent manner. Indeed, this is the first report of transmigration of monocytes across endothelial monolayers to a soluble gradient of secretoneurin. Thus, secretoneurin being released at inflammatory sites such as atherosclerotic lesions may contribute in the pathogenesis of atherosclerosis, as it could induce increased monocyte recruitment from circulating blood into the vessel wall. Additional studies, now already being focused by our laboratory have to investigate further implications of secretoneurin with the vessel wall and its substratum.

The recent model of monocyte migration mimics more closely in vivo situations and also processes employing adhesion of monocyte to the endothelium have to be considered. It is possible that conditions of transendothelial migration to secretoneurin exist in inflammatory reactions or atherosclerotic lesions in vivo, as secretoneurin and other sensory neuropeptides have been detected paravascularly and in the synovium of joints affected by arthritis.

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References

Berthiaume, N., Claing, A., Regoli, D., Warner, T.D., D'Orleans-Juste, P., 1995. Characterization of receptors for kinins and neurokinins in the arterial and venous mesenteric vasculatures of the guinea-pig. Br. J. Pharmacol. 115, 1319–1325.

Butcher, E.C., 1991. Leukocyte–endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 67, 310–314.

Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.M., O'Donohue, T.L., 1985. The anatomy of neuropeptide-Y-containing neurons in rat brain. Neuroscience 15, 1159–1181.

Chuluyan, H.E., Issekutz, A.C., 1993. VLA-4 integrin can mediate CD11/CD18-independent transendothelial migration of human monocytes. J. Clin. Invest. 92, 2768–2777.

Chuluyan, H.E., Issekutz, A.C., 1995. α4-integrin-dependent emigration of monocytes. Springer Semin. Immunopathol. 16, 391–404.

Chuluyan, H.E., Schall, T.J., Yoshimura, T., Issekutz, A.C., 1995. IL-1 activation of endothelium supports VLA-4 (CD49d/CD29)-mediated monocyte transendothelial migration to C5a, MIP-1a, Rantes and PAF but inhibits migration to MCP-1: a regulatory role for endothelium-derived MCP-1. J. Leukocyte Biol. 58, 71–79.

Cybulsky, M.I., Gimbrone, M.A. Jr., 1991. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science (Washington, DC) 251, 788–791.

Cybulsky, M.I., Gimbrone Jr., M.A., 1992. Endothelial expression of a mononuclear leukocyte adhesion molecule in acute inflammation and atherogenesis. In: Simionescu, N., Simionescu, M. (Eds.), Endothelial Cell Dysfunctions. Plenum, New York, pp. 129–140.

Diamond, M.S., Staunton, D.E., de Fougerolles, A.R., Stacker, S.A., Garcia-Aguilar, J., Hibbs, M.L., Springer, T.A., 1990. ICAM-1 (CD54): a counter receptor for Mac-1 (CD11b/CD18). J. Cell. Biol. 111, 3129–3139.

Dunzendorfer, S., Schratzberger, P., Reinisch, N., Kähler, C.M., Wiedermann, C.J., 1998. Secretoneurin, a novel neuropeptide is a potent chemoattractant for human eosinophils. Blood 91, 1527–1532.

Fischer, T., Zumbihl, R., Armand, J., Casellas, P., Rouot, B., 1995. Prolonged elevation of intracellular cyclic AMP levels in U937 cells increases the number of receptors for and the responses to formyl-methionyl-leucylphenylalanine, independently of the differentiation process. Biochem. J. 311, 995–1000.

Furie, M.B., McHugh, D.D., 1989. Migration of neutrophils across endothelial monolayers is stimulated by treatment of the monolayers with interleukin-1 and tumour necrosis factor?. J. Immunol. 143, 3309–3317.

Furie, M.B., Tancinco, M.C.A., Smith, C.W., 1991. Monoclonal antibodies to leukocyte integrins CD11a/CD18 and CD11b/CD18 or intercellular adhesion molecule-1 inhibit chemoattractant stimulated neutrophil transendothelial migration in vitro. Blood 78, 2089–2097.

Gavison, R., Matzner, Y., Fibach, E., 1988. Differential induction of monocytic functions by dibutyryl cyclic AMP and retinoic acid in a human monoblast cell line U937. Isr. J. Med. Sci. 24, 697–701.

Goldstein, I.M., 1992. Complement: biologically active products. In: Gallin, J.I., Goldstein, I.M., Snyderman, R. (Eds.), Inflammation, Basic Principles and Clinical Correlates. Raven Press, New York, pp. 63–83.

Hakkert, B.C., Rentenaar, J.N., Van Aken, W.G., Roos, D., Van Mourik, J.A., 1990. A three dimensional model system to study the interactions between human leukocytes and endothelial cells. Eur. J. Immunol. 20, 2775–2781.

Hirata, Y., Takagi, Y., Takata, S., Fukuda, Y., Yoshimi, H., Fujita, T., 1988. Calcitonin-gene related peptide receptor in cultured vascular smooth muscle and endothelial cells. Biochem. Biophys. Res. Commun. 151, 1113–1121.

Issekutz, A.C., Chuluyan, E., Lopes, N., 1995. CD11/CD18-independent transendothelial migration of human polymorphonuclear leukocytes and monocytes: involvement of distinct and unique mechanisms. J. Leukocyte Biol. 57, 553–561.

Iwamoto, I., Nadel, J.A., 1989. Tachykinin receptor subtype that mediates the increase in vascular permeability in guinea pig skin. Life Sci. 44, 1089–1095.

Jaffe, E.A., Nachmann, R.I., Becker, C.G., Minick, C.R., 1973. Culture of endothelial cells derived from umbilical veins: identification by morphological and immunologic criteria. J. Clin. Invest. 69, 71–77.

Kähler, C.M., Herold, M., Wiedermann, C.J., 1993a. Substance P: a competence factor for human fibroblast proliferation that induces the release of growth-regulatory arachidonic acid metabolites. J. Cell. Physiol. 156, 579–587.

Kähler, C.M., Sitte, B.A., Reinisch, N., Wiedermann, C.J., 1993b. Stimulation of the chemotactic migration of human skin fibroblasts by substance P. Eur. J. Pharmacol. 249, 284–286.

Kähler, C.M., Bellmann, R., Reinisch, N., Schratzberger, P., Gruber, B., Wiedermann, C.J., 1996. Stimulation of human skin fibroblast migration by the neuropeptide secretoneurin. Eur. J. Pharmacol. 304, 135–139.

Kähler, C.M., Schratzberger, P., Kaufmann, G., Wiedermann, C.J., 1997a. Modulation of PECAM-1 cell surface expression, endothelial cell permeability and transendothelial migration of leukocytes in response to secretoneurin in human endothelial cells. Eur. Heart J. 18, 145, (Suppl.).

Kähler, C.M., Kirchmair, R., Kaufmann, G., Kähler, S.T., Reinisch, N., Fischer-Colbrie, R., Hogue-Angeletti, R., Winkler, H., Wiedermann, C.J., 1997b. Inhibition of proliferation and stimulation of migration of endothelial cells by secretoneurin in vitro. Atheroscl. Thromb. Vasc. Biol. 17, 932–939.

Kähler, C.M., Schratzberger, P., Wiedermann, C.J., 1997c. Response of vascular smooth muscle cells to the neuropeptide secretoneurin: a functional role for migration and proliferation in vitro. Atheroscl. Thromb. Vasc. Biol. 17, 2029–2035.

Kew, R.R., Peng, T., DiMartino, S.J., Madhaven, D., Weinman, S.J., Cheng, D., Prossnitz, E.R., 1997. Undifferentiated U937 cells transfected with chemoattractant receptors: a model system to investigate chemotactic mechanisms and receptor/structure/function relationships. J. Leukocyte Biol. 61, 329–337.

Kirchmair, R., Hogue-Angeletti, R., Gutierrez, J., Fischer-Colbrie, R., Winkler, H., 1993. Secretoneurin: a neuropeptide generated in brain, adrenal medulla and other endocrine tissues by proteolytic processing of secretogranin II (chromogranin C). Neuroscience 53, 359–365.

Kirchmair, R., Marksteiner, J., Troger, J., Mahata, S.K., Mahata, M., Donnerer, J., Amann, R., Fischer-Colbrie, R., Winkler, H., 1994. Human and rat primary C-fiber afferents store and release secretoneurin, a novel neuropeptide. Eur. J. Neurosci. 6, 861–868.

Kitayama, J., Carr, M.W., Roth, S.J., Buccola, J., Springer, T.A., 1997. Contrasting response to multiple chemotactic stimuli in transendothelial migration. J. Immunol. 158, 2340–2349.

Leitner, B., Kirchmair, R., Fischer-Colbrie, R., Winkler, H., 1994. Secretoneurin levels in human serum. Neuropeptides 26, 26, (Suppl.).

Moser, R., Schleiffenbaum, B., Groscurth, P., Fehr, J., 1989. Interleukin-1 and tumour necrosis factor stimulate human vascular endothelial cells to promote transendothelial neutrophil passage. J. Clin. Invest. 83, 444–455.

Muller, W.A., Weigl, S.A., Deng, X., Philips, D.M., 1993. PECAM-1 is required for transendothelial migration of leukocytes. J. Exp. Med. 178, 449–460.

Nakagawa, N., Sano, H., Iwamoto, I., 1995. Substance P induces the expression of intercellular adhesion molecule-1 on vascular endothelial cells and enhances neutrophil transendothelial migration. Peptides 16, 721–725.

Payan, D.G., 1992. The role of neuropeptides in inflammation. In: Gallin, J.I., Goldstein, I.M., Snyderman, R. (Eds.), Inflammation: Basic Principles and Clinical Correlates. Raven Press, New York, p. 177.

Randolph, G.J., Furie, M.B., 1995. A soluble gradient of endogenous monocyte chemoattractant protein-1 promotes the transendothelial migration of monocytes in vitro. J. Immunol. 155, 3610–3618.

Rattan, V., Shen, Y., Sulatana, C., Kumar, D., Kalvra, V.K., 1996. Glucose-induced transmigration of monocytes is linked to phosphorylation of PECAM-1 in cultured endothelial cells. Am. J. Physiol. 271, E711–E717.

Reinisch, N., Kirchmair, R., Kähler, C.M., Hogue-Angeletti, R., Fischer-Colbrie, R., Winkler, H., Wiedermann, C.J., 1993. Attraction of human monocytes by the neuropeptide secretoneurin. FEBS Lett. 334, 41–44.

Ross, R., 1993. The pathogenesis of atherosclerosis: a perspective for the 1990's. Nature (London) 362, 801–809.

Ruff, M.R., Wahl, S.M., Pert, C.B., 1985. Substance P receptor mediated chemotaxis of human monocytes. Peptides 6 (2), 107–111, (Suppl.).

Sanabria, P., Silva, W.I., 1994. Specific ^{125}I neuropeptide Y binding to intact cultured bovine adrenal medulla capillary endothelial cells. Microcirculation 1, 267–273.

Saria, A., Troger, J., Kirchmair, R., Fischer-Colbrie, R., Hogue-Angeletti, R., Winkler, H., 1994. Secretoneurin releases dopamine from rat striatal slices: a biological effect of a peptide derived from secretogranin II (chromogranin C). Neuroscience 54, 1–4.

Schneitler, S., Kähler, C.M., Wiedermann, C.J., Hogue-Angeletti, R., Fischer-Colbrie, R., 1998. Specific binding of a ^{125}I-secretoneurin-analogue to human Mono Mac 6 cells. J. Neuroimmunol. 86, 87–91.

Schratzberger, P., Reinisch, N., Kähler, C.M., Wiedermann, C.J., 1996a. Deactivation of chemotaxis of human neutrophils by priming with secretogranin II-derived secretoneurin. Regul. Pept. 63, 65–71.

Schratzberger, P., Wöll, E., Reinisch, N., Kähler, C.M., Wiedermann, C.J., 1996b. Secretoneurin-induced in vitro chemotaxis of human monocytes is inhibited by pertussis toxin and an inhibitor of protein kinase C. Neurosci. Lett. 214, 208–210.

Skerret, P.J., 1990. Substance P causes pain—but also heals. Science 249, 625.

Smith, C.W., Kishimoto, T.K., Abbass, O., Hughes, B., Rothlein, R., McIntire, L.V., Butcher, E., Anderson, D.C., 1991a. Chemotactic factors regulate lectin adhesion molecule-1 (LECAM-1)-dependent neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. J. Clin. Invest. 87, 609–618.

Smith, W.B., Gamble, J.R., Clark-Lewis, I., Vadas, M.A., 1991b. Interleukin-8 induces neutrophil transendothelial migration. Immunology 72, 65–72.

Smith, C.H., Barker, J.N.W.N., Morris, R.W., MacDonald, M.W., Lee, T.H., 1993. Neuropeptides induce rapid expression of endothelial cell adhesion molecules and elicit granulocytic infiltration in human skin. J. Immunol. 151, 3274–3282.

Springer, T.A., 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76, 301–314.

Strubel, N.A., Ngyuen, M., Kansas, G.F., Tedder, T.F., Bischoff, J., 1993. Isolation and characterization of a bovine cDNA encoding a functional homolog of human P-selectin. Biochem. Biophys. Res. Commun. 192, 338–344.

Takahashi, M., Ikeda, U., Masuyama, J.I., Kitagawa, S.I., Kasahara, T., Saito, M., Kano, S., Shimada, K., 1994. Involvement of adhesion molecules in human monocyte adhesion to and transmigration through endothelial cells in vitro. Atherosclerosis 108, 73–81.

Vaudry, H., Colon, J.M., 1991. Identification of a peptide arising from the specific post-translation processing from secretogranin II. FEBS Lett. 284, 31–33.

Wiedermann, C.J., Wiedermann, F.J., Apperl, A., Kieselbach, G., Konwalinka, G., Braunsteiner, H., 1987. In vitro human polymorphonuclear chemokinesis and human monocyte chemotaxis are different activities of amino-terminal and carboxyterminal substance P. Naunyn-Schmiedeberg's Arch. Pharmacol. 340, 665–668.

Wiedermann, F.J., Kähler, C.M., Reinisch, N., Wiedermann, C.J., 1993. Induction of normal human eosinophil migration by substance P. Acta Haematol. 89, 213–215.

Wiedermann, C.J., Schratzberger, P., Kähler, C.M., 1994. Migration of neutrophils across endothelial monolayers is stimulated by treatment of the monolayers with β-endorphin. Brain Behav. Immun. 8, 270–277.

Wiedermann, C.J., Auer, B., Sitte, B.A., Reinisch, N., Schratzberger, P., Kähler, C.M., 1996. Induction of endothelial cell differentiation into capillary-structures by substance P. Eur. J. Pharmacol. 298, 335–338.

Ziche, M., Morbidelli, L., Pacini, N., Gepetti, G., Alessandri, G., 1991. Substance P induces migration of capillary endothelial cells: a novel NK-1 selective receptor mediated activity. Life Sci. 48, 7–11.

Ziegelstein, R.C., Corda, S., Pili, R., Passaniti, A., Lefer, D., Zweier, J.L., Fraticelli, A., Capogrossi, M.C., 1994. Initial contact and subsequent adhesion of human neutrophils or monocytes to human aortic endothelial cells releases an endothelial intracellular calcium store. Circulation 90, 1899–1907.

Zimmermann, B.J., Anderson, D.C., Granger, D.N., 1992. Neuropeptides promote neutrophil adherence to endothelial momolayers. Am. J. Physiol. 263, G678–G682.