Attraction of human monocytes by the neuropeptide secretoneurin

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Secretoneurin is a newly discovered 33-amino-acid peptide derived from secretogranin II (chromogranin C) that is found in sensory afferent C-fibers. We show here that secretoneurin triggers the selective migration of human monocytes in vitro and in vivo. Combinations of secretoneurin with the sensory neuropeptides, substance P or somatostatin, synergistically stimulate such migration. The attraction of monocytes represents the first established function of secretoneurin as a sensory neuropeptide.

Secretoneurin; Secretogranin II; Neurogenic inflammation; Chemotaxis; Monocyte

1. INTRODUCTION

Secretoneurin, a newly discovered 33-amino-acid peptide derived from secretogranin II (chromogranin C), is widely distributed in the central and peripheral nervous and neuroendocrine systems [1,2]. In sensory C-fibers secretoneurin is co-localized with neuropeptides [3] and released from these nerves by capsaicin (manuscript submitted). This localization suggests a possible role of this peptide in neurogenic inflammation. An important step in the inflammatory response to capsaicin is the local accumulation of leukocytes [4]. Here we report that secretoneurin triggers the selective migration of human monocytes in vitro and in vivo. Combinations of secretoneurin with the sensory neuropeptides substance P or somatostatin synergistically stimulate such migration.

2. MATERIALS AND METHODS

Human mononuclear and polymorphonuclear cells were isolated from heparinized forearm venous blood of healthy volunteers and resuspended in RPMI 1640 medium containing 0.5% BSA [5]. Chemotaxis into nitrocellulose micropore filters to test attractants was measured as described [5], using a modified 48-well Boyden chamber (Neuroprobe, Bethesda, MD, USA). A 5-μm pore nitrocellulose filter separates the upper and lower chamber to allow only the actively migrating monocytes or neutrophils to get through the pores. After 90 min and 40 min of incubation at 37°C for monocytes and neutrophils, respectively, the distance of migration of the leading front of cells was measured [6]. In some of the experiments, combinations of test attractants were added to the lower wells of the chambers. Cells were put in the upper compartments. Lyophilized substance P and somatostatin 1–14 (Sigma, St. Louis, MO, USA) were reconstituted to stock solutions of 1 mM in acetic acid (0.2 N) and diluted into assay medium.

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Peptides derived from the primary amino acid sequence of secretogranin II were synthesized by standard t-BOC chemistry and purified by reversed-phase high-performance liquid chromatography (HPLC). In some of the experiments, prior to use, secretoneurin was additionally purified by HPLC gel-filtration chromatography [1].

For measuring skin window migration of human monocytes, a rotating dental stone was used to cause superficial skin abrasions (10 mm in diameter) [7]. An 8-μm pore-sized nitrocellulose membrane (Sartorius Ltd., Göttingen, Germany) was moistened with medium (Hank's balanced salt solution containing 5% human serum albumin), placed over the abrasion and covered with an occlusive dressing. A swab moistened with albumin-containing medium, secretoneurin or Rantes (kindly provided by A.M. Krensky, Stanford Univ. Med. School, Stanford, CA, USA) was placed over the filter. Membranes were changed as described [7] and response rates obtained in serial determinations per field. Cells accumulated in the nitrocellulose membrane were fixed with 37% formaldehyde and stained for monocytes with non-specific esterase activity [8]. The distance of migration towards test attractants and that of medium alone was measured microscopically after 35 min of exposure on forearm lesions of three healthy volunteers. Mean migration of monocytes in the absence of attractants was about 65 μm. The difference between the distance of migration towards test attractants and that of buffer alone is shown. Statistics were calculated with the Mann–Whitney U-test.

3. RESULTS AND DISCUSSION

To test the effect of secretoneurin on the migration of human blood monocytes and neutrophils we performed a series of Boyden chamber assays on these cells. Fig. 1 demonstrates that secretoneurin is a potent attractor of blood monocytes. At a concentration of 100 nM secretoneurin was as effective in attracting monocytes as the positive control N-formylmethionine-leucyl-phenylalanine (fMet-Leu-Phe) at 10 and 100 nM (Fig. 1b). Similar dose–response curves for monocyte migration were observed for rat and human secretoneurin. Human neutrophils were attracted by secre-

toneurin to a small extent and only at high concentrations (1 μM), whereas the positive control fMet-Leu-Phe (10 nM) was highly effective on these cells (Fig. 1a).
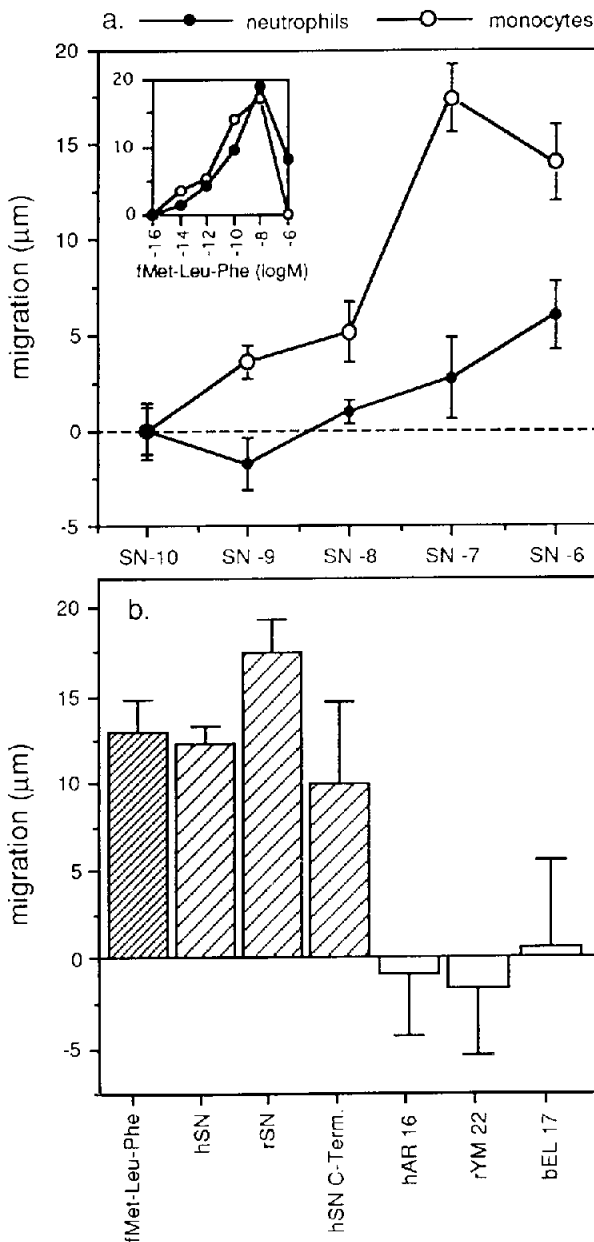

Fig.1. (a) Peripheral blood monocyte and neutrophil migration in response to secretoneurin in vitro. Dose–response curves are shown at log M dilutions. The results are expressed as mean ± S.E.M. of the difference between the distance of migration toward test attractant and that of buffer alone. The mean migration distance of the leading front of cells towards the buffer controls was around 60 μm for monocytes ($n = 7$) and 50 μm for neutrophils ($n = 8$). An identical experiment using fMet-Leu-Phe is given in the insert. (b) Peripheral blood monocyte migration in response to 10 nM of fMet-Leu-Phe, and 100 nM of human [14] secretogranin II 152–184 (human secretoneurin, hSN, $n = 4$), rat [15] secretogranin II 154–186 (rat secretoneurin, rSN, $n = 7$), C-terminal fragment of human secretoneurin (15 amino acids long, $n = 4$), human secretogranin II 1 16 (hAR-16, $n = 3$), rat secretogranin II 568 589 (YM-22, $n = 3$) and bovine [16] secretogranin II 270–286 (EL-17, $n = 3$).

Thus, secretoneurin acts preferentially on blood monocytes.

Stimulation of human monocyte migration was induced by both human and rat secretoneurin as well as by a fragment of human secretoneurin consisting of its 15 C-terminal amino acids (Fig. 1b). Proteolytic cleavage of secretoneurin by 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) completely abolished its chemoattractant activity. As a further control, the effect of a specific antiserum was tested on migration induction by secretoneurin. Secretoneurin-induced attraction at optimal concentration (100 nM, rat) was significantly reduced from 12.5 ± 1.4 μm of migration distance to 1.5 ± 2.2 μm (mean ± S.E.M., $n = 4$; $P = 0.028$, Mann–Whitney U-test) by an immunoglobulin fraction isolated from the antiserum [1]. Migration of monocytes to fMet-Leu-Phe was unaffected by the antibody. Other peptides derived from the precursor secretogranin II, i.e. EL-17, the N-terminus (AR-16) and the C-terminus (YM-22) did not exhibit monocyte chemoattractant activity (Fig. 1b). Thus, chemoattraction is mediated only by secretoneurin and not by any other peptide derived from the precursor secretogranin II. Furthermore, the C-terminal 15 amino acids of secretoneurin are sufficient to promote this activity.

In order to establish whether secretoneurin induces migration of monocytes by acting as a chemokinetic or chemotactic agent, a Zigmond-Hirsch checkerboard analysis was performed (Table I). Secretoneurin potently elicited migration of monocytes when present in excess in the lower compartment of the chemotaxis chambers. Addition of secretoneurin to the cells in the upper compartment did not augment migration. These results are consistent with a chemotactic action [5].

Secretoneurin is co-localized and released with other sensory neuropeptides in C-fiber nerves (see above). To investigate possible interactions of secretoneurin with

Table I

Effect of various concentration gradients of secretoneurin on human peripheral blood monocyte migration into nitrocellulose micropore filters in vitro

		Migration (μm, mean ± S.E.M., $n = 3$) Secretoneurin (M), upper chamber			
		0	10^{-11}	10^{-9}	10^{-7}
Secretoneurin	0	0	6 ± 2.0	5 ± 2.5	−1 ± 3.0
(M), lower	10^{-11}	9 ± 1.1	3 ± 0.3	5 ± 4.0	2 ± 0.9
chamber	10^{-9}	16 ± 2.9	11 ± 1.7	3 ± 1.6	−1 ± 3.2
	10^{-7}	27 ± 8.6	19 ± 2.3	17 ± 5.1	0 ± 4.2

Each value represents the mean ± S.E.M. of the difference between the distance of migration towards test attractants and that of buffer alone after 90 min of incubation at 37°C. Different concentrations of secretoneurin were added to the upper and/or lower compartment of chemotaxis chambers.

other neuropeptides we tested monocyte chemotaxis by secretoneurin in combination with substance P and somatostatin, both of which have also been shown to attract human monocytes [5,9]. A combination of optimal concentrations of substance P or somatostatin with secretoneurin, deactivated the migratory response of monocytes (Fig. 2). This is typical for combinations of high doses of chemoattractants [10]. The mechanism of deactivation of monocyte chemotaxis induced by either a high dose of secretoneurin or by a combination with substance P or somatostatin is not fully understood. For other chemoattractants the same phenomenon was observed and explained with heterologous and homologous desensitization of the receptor or a regulation at a postreceptorial level in the transduction sequence [10,11]. On the other hand, a combination of secretoneurin with substance P or somatostatin at concentrations which failed to induce significant migration of monocytes themselves, acted synergistically on monocyte migration (Fig. 2). Chemoattractants at concentrations that are either ineffective or produce a minor response are known to positively regulate a variety of leukocyte responses to physiologic or pharmacologic

stimuli, termed priming [10]. The observed synergism in monocyte chemotaxis between low doses of secretoneurin and other neuropeptides suggests that secretoneurin also may exert a priming action on the human monocyte response.

A possible interaction of secretoneurin with substance P receptors was tested by the following experiment: at an optimal concentration of 10 nM, substance P attracted monocytes with a potency similar to that of 100 nM of secretoneurin (13.3 ± 1.7 μm and 13.2 ± 1.6 μm, respectively; mean ± S.E.M., n = 5). In the presence of excess concentration of neurokinin-1 receptor antagonist [D-Pro^9-(spiro-γ-lactam)-Leu^{10},Trp^{11}]-Physalaemin 1–11 (Peninsula, Belmont, CA, USA; 1 μM) monocyte migration to substance P (10 nM) was almost totally abolished (0.7 ± 3.0 μm; n = 3; P = 0.046). On the other hand, the response to secretoneurin was not affected (13.8 ± 1.9 μm, n = 5). The neurokinin-2 receptor antagonist [Tyr^5, D-Trp^{6,8,9}, Arg^{10}]-Neurokinin A 4–10 (Peninsula, Belmont, CA, USA; 1 μM) inhibited neither substance P- nor secretoneurin-induced chemotaxis (10.3 ± 0.3 μm and 22.8 ± 2.1 μm, respectively; n = 3). Thus the ability of secretoneurin to induce monocyte
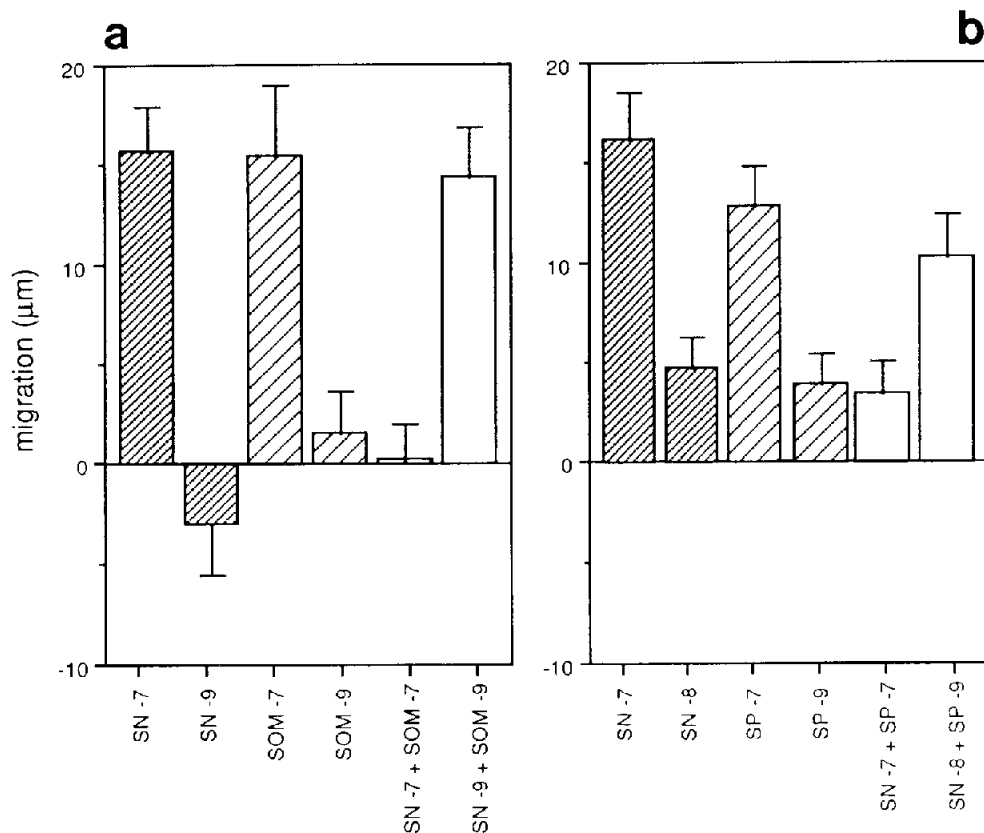

Fig. 2. Peripheral blood monocyte migration in response to combinations of secretoneurin (SN) with somatostatin (SOM) and substance P (SP) respectively, at optimal and suboptimal concentrations (log M). Combination of the peptides at suboptimal concentrations synergistically stimulated migration (a, SN vs. SN plus SOM, and SOM vs. SN plus SOM, P < 0.05, n = 5; b, SN vs. SN plus SP, and SP vs. SN plus SP, P < 0.05, n = 6). Combination at optimal concentrations significantly reduced the chemoattractant effect of the peptides (a, SN vs. SN plus SOM, and SOM vs. SN plus SOM, P < 0.01, n = 5; b, SN vs. SN plus SP, P < 0.005. SP vs. SN plus SP, P < 0.01, n = 6).

Table II

Skin window migration of human monocytes

	Monocyte migration into a skin window (μm)			
	n	Mean	$\pm$ S.E.M.	P value
Rat secretoneurin, 10^{-7} M	9	11.1	1.98	0.046
Human secretoneurin, 10^{-7} M	4	12.5	3.18	0.021
Human secretogranin II				
1–16, 10^{-7} M	3	–2.3	2.33	0.827
Rantes, 10^{-7} M	3	8.7	1.86	0.049

The effect of rat and human secretoneurin on the rate of accumulation and locomotion of human monocytes in response to injury in vivo was investigated. Statistics were calculated with the Mann–Whitney U-test.

attraction does not appear to involve neurokinin-1 or neurokinin-2 receptors.

A human skin window technique using micropore membranes was employed in order to test the effect of secretoneurin on a rapid inflammatory response leading to cellular infiltration of insulted tissue in vivo [7]. The distance of migration of the leading front of monocytes into the filter was measured as a quantitative estimate of the rate of accumulation and locomotion in response to injury. Table II shows that secretoneurin significantly augmented the migration distance of monocytes in vivo. Rantes, a selective monocyte-attracting chemokine [12] similarly affected the migration distance of monocytes, whereas a low concentration of RANTES (1 nM) or the human secretogranin II 1–16 (AR-16) peptide, which did not induce monocyte migration in vitro (Fig. 1b), also failed to stimulate monocyte accumulation in vivo.

Superoxide anion release from adherent mononuclear leukocytes, measured by reduction of ferricytochrome-c [13] could not be induced by 100 nM of secretoneurin after pretreatment of cells for 24 h with either 10% fetal calf serum or with 1000 U/ml of interferon-γ (not shown). Pretreatment of adherent mononuclear leukocytes with 1000 U/ml of interferon-γ significantly activated phorbol 12-myristate 13-acetate (100 ng/ml)-triggered respiration burst activity, whereas 100 nM of secretoneurin failed to prime this effect. Moreover, addition of 100 nM of secretoneurin to 1000 U/ml of interferon-γ did not alter interferon-γ induced priming (not shown).

Our studies show that secretoneurin, a novel sensory neuropeptide, found in afferent C-fibers attracts blood monocytes in a concentration-dependent manner. Application of secretoneurin in vivo to skin lesions enhances monocyte locomotion towards the accumulation at the site of injury. These results suggest that secretoneurin, released from afferent fibers, may participate in neurogenic inflammation and immune system regulation. Secretoneurin can therefore be added to the group of neuropeptides biologically active in the sensory system.

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