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THE PREFERENTIAL HUMAN MONONUCLEAR LEUKOCYTE CHEMOTACTIC ACTIVITY OF THE SUBSTITUENT TETRAPEPTIDES OF ANGIOTENSIN II

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SUMMARY

The amino- and carboxy-terminal substituent tetrapeptides of angiotensin II, Asp-Arg-Val-Tyr and Ile-His-Pro-Phe, elicit substantial human mononuclear leukocyte chemotactic responses *in vitro* that attain maximal levels at tetrapeptide concentrations of 3×10^{-8} M and 3×10^{-7} M, respectively. In contrast, the angiotensin II-derived tetrapeptides evoke only marginal human neutrophil chemotactic responses. Amino acid deletions or substitutions that alter the properties of the tetrapeptides, reduce their chemotactic potency and activity. Limited proteolytic cleavage of angiotensin II thus may convert a pathway with predominantly humoral effects to a source of mediators that regulate cellular immunity and chronic inflammatory responses.

INTRODUCTION

While a role for the octapeptide angiotensin II in the pathogenesis of hypertension and in the development of vascular and myocardial lesions in humans remains a subject of controversy (1-3), there is agreement generally that angiotensin II is a potent constrictor of arterioles and specific segments of capillaries (3,4). The capacity of angiotensin II to increase the permeability of arterial vessels (5,6) and the microvasculature of skin in rats (7,8) appears to be a function of the contraction of the vascular endothelial and smooth muscle cells and the consequent separation of the cell junctions (5-7). The effects of angiotensin II, substituent peptides and some analogues on freely motile cells thus were analyzed in the present study by an assessment of the chemotactic activity of the peptides for human neutrophils and mononuclear leukocytes.

MATERIALS AND METHODS

Chemotactic chambers (Adaps, Inc., Dedham, Mass.), filters with 3 μm or 8 μm pores (Sartorius, supplied by Science Essentials Division of Beckman Instruments, Inc., Wakefield, Mass.), Ficoll-Hypaque and Sephadex G-25 and

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G-75 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), Ultrasphere ODS C-18 reverse-phase high performance liquid chromatography (HPLC)[1] columns (Altex Scientific Co., Inc., Berkeley, Calif.) and reagents for solid-phase peptide syntheses (Beckman Instruments, Inc., Fullerton, Calif.) were obtained as noted. Chemotactic fragments of the fifth component of human complement (C5a) were prepared by filtration on Sephadex G-75 of human sera that had been incubated with zymosan (9). Human angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), human angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe), p-Glu-His-Pro-Gly-NH_2, Asp-Arg-Val-Tyr, and Ile-His-Pro-Phe were purchased from Peninsula Laboratories, Inc., San Carlos, Calif. The latter two peptides and all other peptides employed also were synthesized by solid-phase methods as described (10,11). The peptides were purified by sequential gel filtration on Sephadex G-25 in 0.01 M acetic acid and HPLC on a 4.6 mm x 250 mm C-18 reverse-phase column that was developed with a linear gradient from 0.005 M phosphoric acid (pH 2.2) to 0.005 M phosphoric acid:methanol (1:3, v:v) over 60 min at room temperature (11). The amino acid composition of each purified peptide was determined from an analysis of the free amino acids generated by hydrolysis in 6 M HCl (D-500 Amino Acid Analyzer, Dionex, Inc., Sunnyvale, Calif.) (11).

Human leukocytes from normal subjects were separated by centrifugation on Ficoll-Hypaque into neutrophils of over 96% purity and mononuclear leukocytes containing 17-23% monocytes and 72-82% lymphocytes (9,12). Leukocyte chemotaxis and chemokinesis were assayed by a modification (9,12,13) of the Boyden chamber micropore filter technique utilizing 3 μm pore filters for neutrophils and 8 μm pore filters for mononuclear leukocytes. Chemotaxis was evoked by a stimulus in the lower compartment alone and chemokinesis was elicited by an equal concentration of a stimulus in the lower compartment and in the leukocyte well. The chemotactic and chemokinetic responses were expressed as net leukocytes per high power field (hpf) after subtraction of the background level of migration in the absence of a stimulus. A standard two-sample Student's t-test was used to determine the levels of statistical significance.

RESULTS

Synthetic human angiotensin II, angiotensin III, and the substituent tetrapeptides of angiotensin II elicited human neutrophil chemotactic responses that were maximal in each instance at a peptide concentration of 10^{-7} M and declined at higher concentrations (Fig. 1). In addition, the amino-terminal tetrapeptide substituent of angiotensin II, Asp-Arg-Val-Tyr, exhibited a second peak of neutrophil chemotactic activity at concentrations of 3×10^{-10} M - 10^{-9} M. The magnitude of the mean neutrophil chemotactic responses to the synthetic peptides were only marginal and never exceeded one-fifth of that evoked by an optimal concentration of C5a. In contrast, the mononuclear leukocyte chemotactic responses to each of the same peptides not only were greater than the corresponding neutrophil responses, but achieved maximal levels of up to 50% of that evoked by the C5a (Fig. 1). The mononuclear leukocyte chemo-

[1] Abbreviations used: HPLC, high performance liquid chromatography; hpf, high power field.

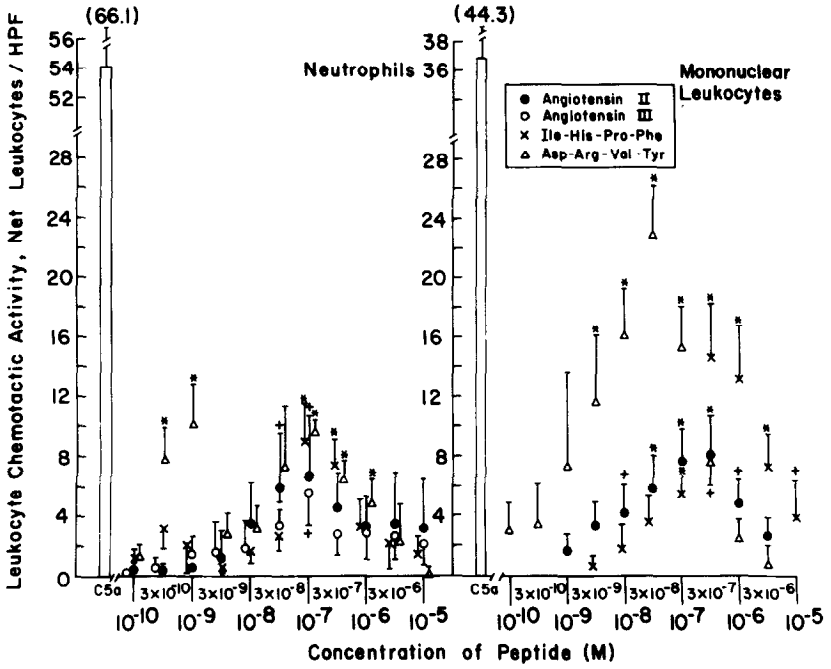

Figure 1 - Human leukocyte chemotactic activity of angiotensin II and substituent peptides. Each point or bar and bracket represents the mean ± S.D. of the results of studies with leukocytes from four different donors. The levels of statistical significance relative to migration in the absence of a stimulus are depicted by symbols: * = $p < 0.01$ and + = $p < 0.05$.

tactic responses to Asp-Arg-Val-Tyr and to Ile-His-Pro-Phe reached maximal levels at concentrations of 3 x 10^{-8} M and 3 x 10^{-7} M, respectively. That the enhanced migration of mononuclear leukocytes exposed to the substituents of angiotensin II represents chemotaxis, rather than chemokinesis, was demonstrated by eliminating the peptide gradients at concentrations of 10^{-8} M - 10^{-6} M, which resulted in mean levels of migration that were only 17-26% of the corresponding chemotactic values in four experiments.

None of the analogues or substituents of the tetrapeptide components of angiotensin II was chemotactically more potent or more active than the native tetrapeptide (Table I). The substitution of asparagine or glutamic acid for aspartic acid in the amino-terminal tetrapeptide had no substantial effect on chemotactic activity. In contrast, substitution of phenylalanine for tyrosine reduced the mononuclear leukocyte chemotactic potency and the

TABLE I

HUMAN NEUTROPHIL AND MONONUCLEAR LEUKOCYTE CHEMOTACTIC ACTIVITY OF ANALOGUES AND SUBSTITUENTS OF ANGIOTENSIN II TETRAPEPTIDES

	Chemotactic Activity			
	Neutrophils		Mononuclear Leukocytes	
Peptide	EC_{max} (M)	Maximal Response (net neutrophils/hpf)	EC_{max} (M)	Maximal Response (net mononuclear leukocytes/hpf)
Asp-Arg-Val-Tyr	10^{-7}	9.5 ± 1.2*	3×10^{-8}	23.0 ± 3.4*
	10^{-9}	10.2 ± 2.6		
Asn-Arg-Val-Tyr	10^{-6}	7.2 ± 3.4	10^{-7}	16.1 ± 2.7
	10^{-8}	8.1 ± 3.9		
Glu-Arg-Val-Tyr	10^{-7}	12.5 ± 4.0	3×10^{-8}	19.2 ± 3.6
	10^{-9}	10.8 ± 3.6		
Asp-Arg-Val-Phe	10^{-6}	6.3 ± 2.2	3×10^{-7}	10.3 ± 4.4
	10^{-8}	5.9 ± 2.4		
Lys-Arg-Val-Gly	10^{-5}	3.7 ± 1.1	10^{-5}	4.0 ± 2.2
Asp-Arg-Val	10^{-6}	4.4 ± 2.9	10^{-6}	3.6 ± 1.7
Arg-Val-Tyr	3×10^{-6}	4.9 ± 2.4	10^{-5}	4.3 ± 2.1
Ile-His-Pro-Phe	10^{-7}	9.0 ± 2.6	3×10^{-7}	14.6 ± 3.7
Val-His-Pro-Phe	3×10^{-7}	6.4 ± 2.1	3×10^{-7}	10.9 ± 4.3
p-Glu-His-Pro-Gly-NH_2	10^{-7}	4.1 ± 2.2	10^{-6}	5.7 ± 2.4
Ile-His-Pro	3×10^{-7}	2.3 ± 1.5	10^{-6}	3.9 ± 2.7
His-Pro	10^{-6}	1.8 ± 0.9	10^{-5}	1.3 ± 1.6
His-Pro-Phe	3×10^{-6}	2.5 ± 1.8	10^{-5}	1.5 ± 0.9
Ile-Pro-Phe	3×10^{-7}	4.6 ± 1.3	10^{-6}	5.2 ± 2.8

* Each value is the mean ± S.D. of the results of studies with leukocytes from four different donors, except for Asn-Arg-Val-Tyr (n=3), Asp-Arg-Val-Phe (n=3), and Ile-Pro-Phe (n=3). EC_{max} = the minimal effective concentration required to achieve a maximal chemotactic response.

maximal activity by about one-half, while substitution for both aspartic acid and tyrosine or deletion of either residue eliminated the chemotactic activity. Similarly, the substitution of valine for isoleucine in the carboxy-terminal tetrapeptide had no effect on chemotactic activity, while substitution for both isoleucine and phenylalanine or deletion of any residue essentially eliminated the chemotactic activity for both types of leukocytes (Table I).

DISCUSSION

The amino-terminal and carboxy-terminal tetrapeptide substituents of angiotensin II stimulate mononuclear leukocyte chemotaxis which reaches maximal levels of approximately 50% and 30%, respectively, of that evoked by optimal concentrations of C5a at tetrapeptide concentrations of 3×10^{-8} M and 3×10^{-7} M (Fig. 1). In contrast, the maximal neutrophil chemotactic responses elicited by the same tetrapeptides and by angiotensins II and III never exceed 15-20% of the response to C5a. The preferential chemotactic activity of the tetrapeptides of angiotensin II for mononuclear leukocytes is shared only by native collagen (14), by specific peptide products of the proteolysis of collagen (14), fibrinogen (15), and elastin (16), by desmosine (16), and by a lymphokine-like principle secreted by lymphocytes that have been exposed to mitogen (17). That the substituent tetrapeptides are more active chemotactic factors than the parent octapeptide, angiotensin II, suggests that selective cleavage of angiotensin II would convert the predominantly humoral effector pathway to a source of mediators that promote the influx of leukocytes characteristic of chronic inflammation.

The structural determinants of the mononuclear leukocyte chemotactic activity of the tetrapeptides have not been elucidated definitively, but are not contained in tripeptide components and require specific amino- and carboxy-terminal residues for optimal expression (Table I). It also has not been established whether a relationship exists between the chemotactic activity of the tetrapeptides and their ionophoric properties (18) or capacity to contract vascular endothelial cells (5-7). It is of interest that the amino-

terminal tetrapeptide substituent Asp-Arg-Val-Tyr, which is the most active mononuclear leukocyte chemotactic factor, exhibits significant structural homology with the pentapeptide Arg-Lys-Asp-Val-Tyr, that is the functionally critical constituent of the thymic hormone, thymopoietin (19). The pentapeptide, like thymopoietin, simultaneously induces the differentiation of murine prothymocytes to thymocytes and suppresses the development of B lymphocytes. Further studies with purified subpopulations of mononuclear leukocytes will be required to determine the effects of the Asp-Arg-Val-Tyr substituent of angiotensin II on lymphocytes as compared to monocytes.

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