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Activation of human peripheral monocytes by angiotensin II

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Abstract

This study has investigated the ability of the vasoconstrictor peptide angiotensin II to activate human peripheral blood monocytes. Activation was monitored by measuring both the release of tumor necrosis factor α from monocytes and their adhesion to monolayers of human endothelial cells. Angiotensin II-elicited activation of monocytes was dose-dependent (half-maximally effective concentration ≈ 0.2 nM), saturable (maximally effective concentration ≈ 5 nM), and sensitive to inhibition by the angiotensin type 1 receptor antagonist ZD 7155. Such direct actions imply that angiotensin II is an important candidate stimulus for the subendothelial infiltration of monocytes observed in atherogenesis and hypertension.

Key words: Monocyte; Angiotensin II; AT1 receptor; Activation; Adhesion; Tumor necrosis factor α

1. Introduction

Endothelial dysfunction and/or injury is one of the earliest events postulated to occur in the genesis of the atherosclerotic lesion [1]. This is followed by a series of events which include monocyte recruitment and macrophage formation, transendothelial migration of monocytes macrophages, lipid deposition, vascular smooth muscle cell proliferation and synthesis of extracellular matrix [1]. Subendothelial monocyte/macrophage invasion of the vessel wall requires activation of peripheral blood monocytes and their specific interaction with the endothelial cell surface [2]. Thus, atherosclerotic lesions occur mainly in regions of blood vessels where rheologic conditions are optimal (i.e. low circulatory shear stress) for endothelium–monocyte contact and monocyte transmigration [5]. The endothelial dysfunction and monocyte macrophage infiltration that occurs both in hypertension and with aging also occur preferentially in vessel locations exposed to slow shear forces [3,4]. In the aorta and carotid artery of spontaneously hypertensive rats both subendothelial infiltration of mononuclear cells and endothelial dysfunction can be decreased by treatment of animals with angiotensin converting enzyme inhibitors (ACEI) [6]. These concomitant beneficial effects of ACEI have been proposed to result from either a primary action of ACEI on endothelial function (e.g. via decreased bradykinin degradation and increased EDRF release) with secondary prevention of monocyte/macrophage infiltration, or vice versa [6]. Other than blood pressure lowering effects, the mechanisms underlying primary actions of ACEI on monocyte/macrophage infiltration are unclear. To explore the possibility that angiotensin II (Ang II) might be directly involved in the process of monocyte recruitment, we have investigated whether Ang II receptors are expressed by human peripheral blood monocytes and whether Ang II is capable of promoting monocyte activation and adhesion.

2. Experimental

2.1. Isolation and activation of peripheral blood monocytes and tumor necrosis factor (TNF) α measurements

All solutions and chemicals used in isolation and activation procedures were endotoxin-free (endotoxin < 0.01 ng/ml). Human peripheral blood monocytes were isolated using the continuous Percoll gradient procedure exactly as described by Gmelig-Meyling and Waldmann [7]. Cells were finally resuspended and maintained in RPMI 1640 medium supplemented with 5% human AB serum, 15 μg/ml gentamycin and 10 μg/ml polymyxin B, and 10 mM each of sodium pyruvate, non-essential amino acids, sodium bicarbonate and HEPES (pH 7.4). The relative amount of monocytes and lymphocytes was determined using the Diffquick staining procedure (Merz and Dade). Monocyte suspensions were transferred into serum-free (5% human serum albumin instead of 5% human AB serum) supplemented medium (cell count adjusted to 5×10^5 cells/ml) at least 2 h before all experimental protocols. Monocyte activation by endotoxin (bacterial lipopolysaccharide (LPS)) or Ang II was determined by measuring the release of TNFα into the culture supernatant (EIA assay, threshold sensitivity 0.05 ng TNFα; anti-TNFα antibodies provided by Hoffmann LaRoche Ltd., Basel, Switzerland).

2.2. RNA isolation, RT-PCR, Southern analyses and probes

Isolation of total RNA was performed according to Chomczynski [8], and preparations were treated with RNase-free DNase. Reverse transcriptase reactions were performed according to standard procedures using AMV reverse transcriptase (Boehringer, Mannheim, Germany) and 2 μg of total RNA. Single-stranded cDNA corresponding to 200 ng of total RNA was used in each PCR reaction. For PCR, Perkin Elmer machinery and reagents were used according to the suppliers protocols. The oligonucleotides used correlated to regions adjacent to the entire coding region of the human angiotensin AT_1 type receptor: antisense TTATTGATTCACTCTTCTAC; sense CGAACATGTCACTCAACCTC. Primers and conditions for cycling were selected using the Oligo program for Macintosh. 30 cycles with the following parameters were performed: 1 min each denaturation at 97°C, annealing at 55°C and synthesis at 72°C. The identity of the amplificates generated was verified by partial sequencing and/or Southern blotting using a homologous partial, nested cDNA probe according to standard protocols.

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2.3. Adhesion of monocytes to endothelial cell monolayers

Human umbilical vein endothelial cells (HUVEC; ATCC CRL 1730) were grown and maintained in supplemented RPMI 1640 medium (as above but with 10% human AB serum) under endotoxin-free conditions. Confluent cultures of HUVEC were changed to serum-free medium conditions (10% human AB serum replaced by 5% human serum albumin) at least 2 h before adhesion experiments. Monocytes (in serum-free supplemented medium) were activated by exposure for 6 h to either LPS or Ang II (10^{-8} M). In some experiments monocytes were preincubated for 30 min with the AT_1 receptor antagonist ZD 7155 (10^{-8} M) before exposure to Ang II. Monocytes continuously maintained in serum-free supplemented medium served as negative controls. Following activation protocols, monocytes were washed once in serum-free medium and then seeded (at 5×10^5 cells/well and into triplicate wells for each experimental point) onto confluent HUVEC monolayers. After 2 h, non-adherent monocytes were collected and counted. Monocyte adhesion is given as the number of adherent cells expressed as the percent of that number initially seeded (100%).

3. Results

Total RNA from human peripheral blood monocytes and human vascular smooth muscle cells (as positive control) was subjected to RT-PCR using oligonucleotides specific for the human angiotensin type 1 (AT_1) receptor. Both monocytes and smooth muscle cells expressed AT_1-specific transcripts (Fig. 1). The result was confirmed by Southern Blot analysis (Fig. 1) and partial sequencing of the amplification products (not shown).

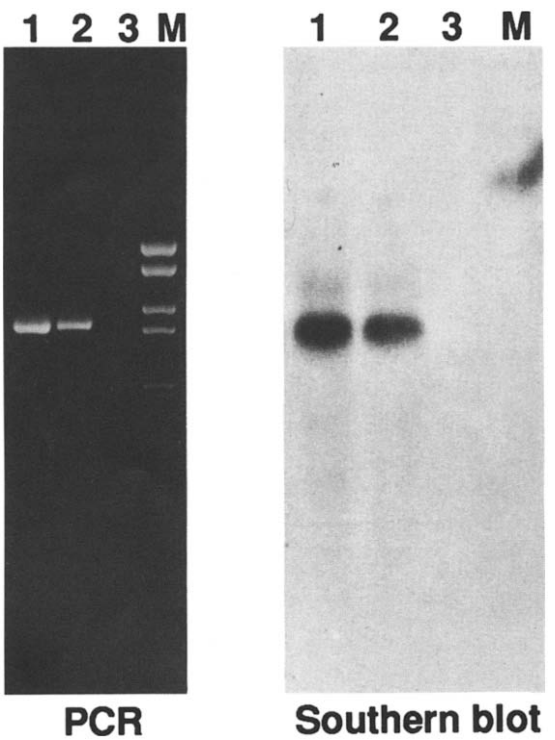

Fig. 1. Expression of angiotensin AT_1 receptor transcripts in human monocytes and vascular smooth muscle cells. RT-PCR and Southern blot of amplificates of total RNA from peripheral blood monocytes (lane 2), cultured aortic smooth muscle cells (lane 1; positive control) and CHO cells (lane 3; negative control). M = DNA molecular weight marker. Amplificates were hybridized to a radiolabelled *Acc*I–*Hin*dIII subfragment of the human AT_1 receptor cDNA as a probe. Methods are detailed in section 2.

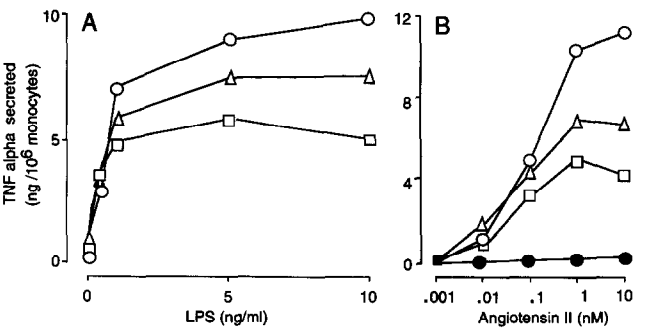

Fig. 2. Secretion of TNFα by human peripheral blood monocytes. Dose-dependent stimulation of monocytes by either (A) LPS or (B) angiotensin II either alone (open symbols) or after preincubation with 10 nM ZD 7155 (filled symbols). LPS- and Ang II-induced TNFα secretion responses of monocytes from three separate donors are presented (1 = ○, 2 = △, 3 = □). ZD 7155 completely inhibited Ang II-induced TNFα secretion in monocytes from the three different donors (● = 1, 2 and 3). Data points represent mean values (S.D. < 10%) from triplicate measurements. All methods are detailed in section 2.

The activation of blood moncytes by Ang II or LPS (as the positive reference) was monitored by measuring the release of TNF α into culture supernatant. LPS dose-dependently induced release of TNF α from monocytes (Fig. 2A). The maximal amounts of TNF α released varied between monocyte preparations from different donors (Fig. 2A). Ang II also induced secretion of TNF α from blood monocytes (Fig. 2B). This response was dose-dependent (half-maximally effective concentration of ≈ 0.2 nM) and saturable (maximally effective concentration ≈ 1 nM), and as in the case of LPS-induced monocyte activation, monocytes from different donors exhibited different maximal activation responses to Ang II (Fig. 2B). Ang II-induced release of TNF α was completely inhibited in monocytes preincubated with the AT_1 receptor antagonist, ZD 7155 (10 nM) (Fig. 2B).

Compared to resting monocytes (<10% adhesion), Ang II-activated monocytes exhibited high adhesion (≈70%) to monolayers of HUVEC (Fig. 3). In monocytes preincubated with the AT_1 receptor antagonist before Ang II treatment, adherence was not significantly different from controls (Fig. 3).

4. Discussion

'Endothelial injury' may be initiated by rheological parameters, and low shear stress has been demonstrated to be greatly conducive toward leucocyte adhesion to endothelial cells [3,9]. However, adherent/subendothelial monocytes in capacitance arteries of the spontaneously hypertensive rat exhibit a heterogenous regional distribution, namely present in the aorta and carotid artery but absent in the renal artery [6]. Furthermore, although

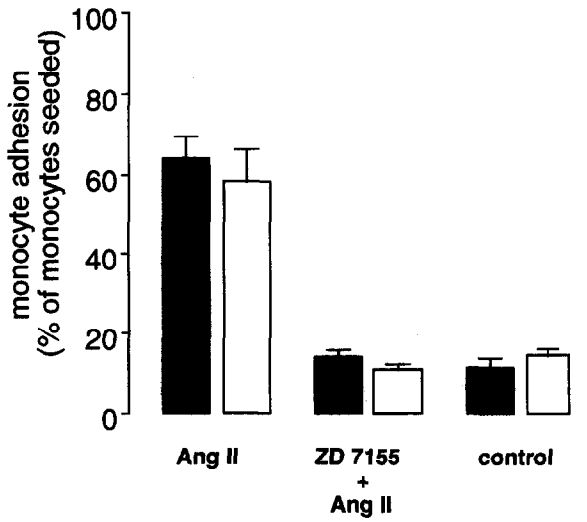

Fig. 3. Adhesion of human monocytes to human umbilical vein endothelial cells. Monocytes were incubated for 6 h in the presence of either 5% human serum albumin (control) or Ang II (10^{-8} M) with or without a 30 min preincubation with ZD 7155 (10^{-8} M), and then tested for adhesion to HUVEC. All protocols are described in section 2. Results (mean ± S.D. from triplicate experiments) on monocytes from two donors (2 and 3 as in Fig. 2) are presented.

both ACEI and hydralazine lower blood pressure, only ACEI effectively reduces monocyte macrophage infiltration and endothelial dysfunction [6,10]. It was thus proposed that the trigger of monocyte/macrophage adhesion stems rather from the vessel wall itself than from mere rheological parameters [6]. Our present report of Ang II-mediated activation of peripheral blood mononuclear cells and increased adhesion of Ang II-activated monocytes to human endothelial cells is in support of such a proposal.

We have demonstrated that Ang II is capable of activating peripheral blood monocytes in a manner that is dose-dependent, saturable and sensitive to inhibition by AT_1 receptor antagonism. These observations, together with the expression of AT_1 receptor transcripts by monocytes, suggests that Ang II-induced activation of monocytes occurs via a receptor-mediated mechanism. The TNFα secretion response of monocytes to Ang II occurred at physiologically relevant concentrations of Ang II (10^{-10}–10^{-9} M), and at maximum was quantitatively comparable to endotoxin (LPS)-induced TNFα release from monocytes. TNFα has been demonstrated to exert a direct toxic effect on cultured vascular endothelial cells [11], and thus in vivo, the activation of monocytes by Ang II may initiate and/or perpetuate a status of 'endothelial injury'.

In vivo the vascular entothelial cell itself is the major source of Ang II [12]. Under normal conditions, endothelium-derived Ang II is secreted towards the cell layers of the tunica media rather than the luminal side of the vessel, and this is reflected in minimal amounts of circulating Ang II [13]. However, with age and in hypertension, there is clear evidence for not only an elevated activity of angiotensin generating systems [13], but also elevated levels of circulating Ang II and a loss of endothelial cell polarity [14]. The concomitant beneficial effects of ACEI treatment on monocyte adhesion/transmigration and endothelial dysfunction in hypertension may therefore be explained by inhibition of Ang II generation by ACEI and the resultant reduction of monocyte activation by this peptide. Direct prevention of Ang II-mediated monocyte activation by AT_1 receptor antagonism may provide a valuable therapeutic avenue for prevention of 'endothelial activation/injury' in both atherosclerosis and hypertension.

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References

[1] Badimon, J.J., Fuster, V., Chesebro, J.H. and Badimon, L. (1993) Circulation 87, II-3–II-16.
[2] Poole, J.F.C. and Florey, H.W. (1988) J. Pathol. Bacteriol. 75, 245–252.
[3] Ku, D.N., Giddens, D.P., Zarins, C.K. and Glagov, S. (1985) Arteriosclerosis 5, 293–302.
[4] Linder, L., Kiowski, W., Bühler, F.R. and Lüscher, T.F. (1990) Am. J. Hypertens. 3, 55–58.
[5] Ross, R. (1993) Nature 362, 801–809.
[6] Clozel, M., Kuhn, H., Hefti, F. and Baumgartner, H.R. (1991) Hypertension 18, 133–141.
[7] Gmelig-Meyling, F. and Waldmann, T.A. (1980) J. Immunol. Methods 33, 1–9.
[8] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[9] Chobanian, A.V., Forney-Prescott, M. and Haudenschild, C.C. (1984) Exp. Mol. Pathol. 41, 153–169.
[10] Clozel, M., Kuhn, H. and Hefti, F. (1990) Hypertension 16, 532–540.
[11] Hicks, C., Breit, S.N. and Penny, R. (1989) Immunol. Cell. Biol. 67, 271–277.
[12] Doyle, A.E. and Bearn, A.G. (1984) Hypertension and the Renin–Angiotensin System: Therapeutic Approaches, Raven Press, New York.
[13] Dzau, V.J. and Pratt, R.E. (1993) In: The Renin-Angiotensin system (J.I.S. Robertson and M.G. Nicholis, eds. pp. 42.1–42.8, Gower Medical, London.
[14] Panza, J.A., Quyyumi, A.A., Brush, J.E. and Epstein, S.E. (1990) N. Engl. J. Med. 323, 22–27.