ACE Inhibition Lowers Angiotensin-II-Induced Monocyte Adhesion to HUVEC by Reduction of p65 Translocation and AT1 Expression

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Key Words

Angiotensin II · ACE inhibitor · Inflammation · Atherosclerosis · Angiotensin receptor

Abstract

Angiotensin-converting enzyme (ACE) inhibitors interfere with several key events of vascular inflammation resulting in impressive reductions in coronary vascular events. However, in human arteries ACE inhibitors block the production of angiotensin II (AngII) incompletely because of the involvement of alternative pathways in local AngII formation. Therefore, our study concentrated on the presumed modulation by ACE inhibition of local AngII-mediated inflammatory actions by a mechanism independent of blockage of AngII formation. We analyzed the effect of the ACE inhibitor ramiprilat on AngII-dependent cell adhesion molecule (CAM) expression and adhesion of monocytic THP-1 cells to endothelial cells. AngII induced upregulation of P-selectin, VCAM-1 and ICAM-1 on endothelial cells via activation of AT1, which was correlated with enhanced THP-1 adhesion in flow chamber assays. Both enhanced adhesion and adhesion molecule expression were significantly reduced by pretreatment with ramiprilat. Ramiprilat reduced AT1 expression on endothelial cells and decreased the AngII-

induced p65 translocation into the nucleus. Diminished AT1 expression and adhesion molecule expression in response to ramiprilat treatment were partially reversed after incubation with a bradykinin 2 receptor antagonist, suggesting that elevated bradykinin levels under ACE inhibition may be involved in the beneficial effect of ACE inhibitors. Thus, modulation of the local AngII system by ramiprilat may at least in part contribute to the benefits of ACE inhibition in the treatment of atherosclerotic diseases.

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Introduction

Local inflammatory actions play a key role in the initiation and progression of atherosclerosis which will finally lead to clinical complications such as myocardial infarction and stroke [1, 2]. Angiotensin II (AngII), the main effector of the renin-angiotensin system (RAS), is known to be involved in various proinflammatory actions in the vascular wall, including adhesion molecule, chemokine and cytokine expression, and generation of reactive oxygen species [3]. Nuclear factor NF- κ B is considered to be an important link between AngII and its inflammatory actions. Binding of AngII to the AngII receptor 1

(AT1) results in activation of four different signaling pathways, of which at least three are known to activate NF- κ B: activation of NADH/NADPH oxidase, activation of tyrosine kinase, and Gq uncoupling [4]. After activation, the NF- κ B heterodimer consisting of p50 and p65 translocates from the cytoplasm into the nucleus where it binds target genes and stimulates transcription of specific sets of genes relevant to the pathophysiology of the vessel wall [5]. The AngII-mediated stimulation of NF- κ B via AT1 leads to upregulation of proinflammatory cytokines, chemokines and adhesion molecules [3, 6].

Dzau [7] has documented the existence of both a local and a systemic RAS. While short-term effects of cardiovascular homeostasis such as vasoconstriction and hypertension can be ascribed to the systemic RAS, long-term effects such as inflammatory actions in the vascular wall relate to functions of the local RAS. Angiotensin-converting-enzyme (ACE)-like enzymes such as cathepsin G, cathepsin D, tonin and chymase were identified locally in the vessel wall [3]. In particular, chymase plays an important role in local AngII formation in the atherosclerotic plaque. In the human aorta, 80–90% of the AngII-forming activity is chymase-dependent [8]. Chymase is mainly mast cell derived [9], while cathepsin G is expressed in neutrophils and monocytes [10]. The local accumulation of high levels of AngII, produced by ACE and alternative pathways of AngII production, activates AngII receptors on different cell types, leading to progressive lesion formation via proliferation of smooth muscle cells, formation of foam cells, and facilitation of thrombosis.

Previous studies have revealed that not all the potential anti-atherosclerotic actions of ACE inhibitors are shared by AT1 antagonists. Therefore, we suspected that additional mechanisms independent of the inhibition of AngII generation might be involved in the anti-atherosclerotic actions of ACE inhibitors. Results by Berkenboom et al. [11] showed that ramipril but not losartan could prevent ox-LDL-induced endothelial damage in Watanabe hypercholesterolemic rabbits. In hypercholesterolemic rabbits ACE inhibitors were found to be more effective than losartan in improving vascular function [12].

We therefore hypothesized that ACE inhibitors, in spite of the incomplete inhibition of tissue AngII formation, can modulate the local vascular AngII-mediated inflammatory action by influencing the inflammatory signal cascade in AT1-bearing cells of the atherosclerotic plaque. To this end, effects of the ACE inhibitor ramiprilat on the expression of endothelial cell adhesion molecules

and on the adhesion of THP-1 cells to AngII-stimulated human umbilical vein endothelial cells (HUVEC) were analyzed.

Materials and Methods

Cell Culture

Monocytic THP-1 cells were cultured in RPMI-1640 (Biochrom, Germany) containing 10% FCS (Biochrom) at a density of up to 1×10^6 cells/ml. HUVEC were isolated and cultured as described previously [13]. Endothelial cells were growth factor starved 24 h prior to stimulation with AngII. Ramiprilat, a gift from Aventis (Frankfurt, Germany), was dissolved at 1 mM in PBS and stored at -20°C until use. Losartan, PD123.319, AngII, HOE-140, des-Arg⁹, [Leu⁸]-bradykinin, captopril, and lipopolysaccharide (LPS) were purchased from Sigma (Munich, Germany). Simvastatin was a kind gift from Merck (Darmstadt, Germany).

Flow Cytometry

After stimulation of HUVEC with AngII (1 μM , 6 h) and when indicated with ramiprilat (1 μM , 20 h prior to AngII), losartan (1 μM , 30 min prior to AngII) or PD123.319 (1 μM , 30 min prior to AngII) cells were detached with accutase, washed with PBS and then incubated with 0.5% BSA in PBS for 30 min at 4°C . HUVEC were stained with the respective antibody for 30 min at 4°C . Anti-human CD106 PE (CBL 206P), anti-human CD62E PE (CBL180P) (Cymbus, UK), anti-human CD62P FITC, anti-human CD54 PE, mouse IgG1 PE isotype, mouse IgG1 FITC isotype (BD Biosciences, USA) were used. After two washes in PBS, cells were immediately assayed in Becton Dickinson FACS Calibur (Becton Dickinson, USA). Results are expressed as mean fluorescence intensity (MFI).

For analysis of angiotensin II receptor expression on HUVEC, cells were washed twice with PBS after treatment as indicated, incubated with 0.5% BSA in PBS for 30 min at 4°C . 1 $\mu\text{g}/\text{ml}$ of either anti human AT1 (306, SantaCruz), anti human AT2 (H-143, SantaCruz) or rabbit IgG (Sigma) was added for 30 min at 4°C . After washing, cells were incubated with mouse anti-rabbit-FITC conjugated antibody (Sigma), washed again and assayed in Becton Dickinson FACS Calibur (Becton Dickinson).

Flow Chamber Analysis

Laminar flow chamber assays were performed on confluent HUVEC. Confluent endothelial cells were treated with AngII, ramiprilat, PD123.319 or losartan as described above. LPS at a concentration of 1 $\mu\text{g}/\text{ml}$ for 4 h was used as positive control. HUVEC were washed twice with PBS before cell injection to wash off excessive reagents. Flow chamber experiments were performed with THP-1 cells (10^6 cells/ml) resuspended in medium containing 1% FCS and infused at 2 dyne/cm² for 4 min. The experiments were recorded with a CCD camera (Nikon DXM 1200, Düsseldorf, Germany) for off-line analysis. The analysis was performed by counting the number of adherent cells in four separate fields in each well.

Western Blotting

After treatment of HUVEC with ramiprilat or simvastatin, cells were washed with PBS and lysed with 0.1 ml/ 10^6 cells of lysis buffer (50 mM HEPES, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA,

Fig. 1. AngII enhances the expression of ICAM-1 (a), VCAM-1 (b), P-selectin (c) but not E-selectin (d) on HUVEC. HUVEC were stimulated with AngII (1 μ M, 6 h) and when indicated with losartan (1 μ M) or PD123.319 (1 μ M). Values are given as MFI (mean $\pm$ SD) in percent of basal expression, which was set to 100% in each experiment. n = 7, * p < 0.05, ** p < 0.01; n.s. = not significant.

10% glycerol, 1 mM PMSF, 1.9 mg/ml aprotinin, 0.5 mg/ml leupeptin, pH 7.4). Following a protein concentration assay, the proteins (30 μ g per lane) were separated by 10% SDS-PAGE under reducing conditions and transferred to a PVDF membrane. Non-specific binding was blocked by 10% dry milk in TBS-Tween (10 mM Tris, 100 mM NaCl, 0.1% Tween-20, pH 7.5) overnight at 4°C. Incubation with anti-AT1 (1:500, Santa Cruz, Germany, clone 306) or anti-AT2 antibody (1:500, Santa Cruz, clone H-143) was followed by incubation with horseradish peroxidase-conjugated goat anti-mouse antibody (1:5,000, Amersham, UK). Staining was visualized by an enhanced chemiluminescence system (Amersham). To confirm equal protein loading, membranes were stained with Ponceau S. The relative amount of AngII receptor expression was estimated using densitometric analysis (Quantity One, BioRad, Germany).

p65 Translocation Assay

The assay was performed as described previously [14]. Briefly, after stimulation HUVEC were harvested and washed with PBS. Nucleus preparations were obtained by incubating the cells with 200 μ l Pipes-Triton buffer (10 mM Pipes, 0.1 M NaCl, 2 mM MgCl₂ and 0.1% Triton X-100 in H₂O) for 30 min at 4°C. Thereafter nuclei were stained with mouse anti-human NF- κ B p65 monoclonal antibody (Santa Cruz, clone F-6) or with the matching isotype (DakoCytomation, Germany, X0931) at 5 μ g/ml for 30 min.

After washing, the nuclei were incubated with a secondary FITC-conjugated goat anti-mouse antibody (DakoCytomation, F0479) for an additional 30 min at 4°C. The nuclei were then counterstained with propidium iodide (PI, BD Biosciences) at 1 μ g/ml and immediately analyzed using FACS.

Statistics

Data are presented as mean $\pm$ SD and were analyzed by analysis of variance (ANOVA). When significant main effects were observed, a Tukey post hoc test for multiple comparisons was performed. The level of significance set for the investigation was p < 0.05.

Results

AngII Induces Monocyte Adhesion to HUVEC via AT1-Mediated Adhesion Molecule Expression

Adhesion molecule expression on HUVEC was analyzed by FACS upon stimulation with AngII (1 μ M) for 6 h. In comparison with unstimulated cells, we found a clear-cut increased expression of ICAM-1 (p < 0.01, fig. 1a), VCAM-1 (p < 0.05, fig. 1b) and P-selectin (p < 0.05,

Fig. 2. Effect of AngII on THP-1 adhesion to HUVEC. THP-1 cells were perfused over AngII-treated ($1 \mu\text{M}$, 6 h) HUVEC monolayers for 4 min and the number of adherent cells in four different fields was subsequently counted. Losartan ($1 \mu\text{M}$) and PD123.319 ($1 \mu\text{M}$) were added 30 min before AngII stimulation. Adhesion of THP-1 cells to HUVEC under different conditions is expressed as adherent cells/mm². Results are given as mean $\pm$ SD, * $p < 0.01$, $n = 6$.

fig. 1c) but not E-selectin ($p = 0.7$, fig. 1d). Enhanced expression of adhesion molecules upon AngII stimulation was blocked by pretreatment with the AT1 antagonist losartan but not the AT2 antagonist PD123.319, indicating an AT1-dependent mechanism to be responsible for the induction. The stimulation over 6 h was found to be more effective than treatment for 4 or 24 h (data not shown). However, significant induction of E-selectin could not be detected at any time, although stimulation with LPS ($1 \mu\text{M}$, 4 h) resulted in a markedly increased expression of this molecule (data not shown).

To demonstrate a functional correlate to the enhanced adhesion molecule expression, flow chamber assays were performed. THP-1 cells were perfused at 2 dyne/cm^2 over stimulated HUVEC and the number of adherent cells was analyzed in four different fields. Treatment of HUVEC with AngII resulted in a significant enhancement of THP-1 adhesion ($p < 0.001$, fig. 2) which was almost completely blocked by losartan. PD123.319 did not signifi-

cantly alter the adhesion compared to AngII-treated cells.

Ramiprilat Reduces AngII-Induced Expression of Adhesion Molecules and Adhesion of Monocytes

We further investigated the effect of the ACE inhibitor ramiprilat in our study. In HUVEC not stimulated with AngII, the adhesion molecule expression was not significantly influenced by ramiprilat (data not shown). In contrast, in AngII-stimulated HUVEC ($1 \mu\text{M}$, 6 h), pretreatment with ramiprilat ($1 \mu\text{M}$, 20 h) caused a significant reduction in adhesion molecule expression as compared with cells stimulated with AngII only (fig. 3a). Similarly, HUVEC treated with LPS ($1 \mu\text{g/ml}$, 4 h) resulted in increased ICAM-1 expression, which was significantly attenuated by 45% after pretreatment with ramiprilat ($1 \mu\text{M}$, 20 h) (data not shown).

Since enhanced adhesion molecule expression is associated with increased THP-1 adhesion to endothelium, we analyzed if ramiprilat-induced decrease in adhesion molecule expression also resulted in reduced monocyte adhesion. Therefore, HUVEC were treated with ramiprilat ($1 \mu\text{M}$) for 20 h prior to stimulation with AngII. The ACE inhibitor caused a significant reduction in THP-1 adhesion to endothelial cells ($p < 0.01$, fig. 3b).

Ramiprilat Reduces AngII-Mediated p65 Translocation in HUVEC

As ramiprilat was effective despite AngII stimulation, the ACE inhibitor had to interact with the RAS distal from AngII formation. We therefore analyzed the p65 translocation and the AT1 expression on HUVEC as possible mechanisms responsible for the reduced THP-1 adhesion. AngII-dependent increase in adhesion molecule expression can be mediated intracellularly by increased activity of NF- κB , which results in enhanced p65 translocation into the nucleus. Before the measurement of p65 translocation, HUVEC were stimulated with $1 \mu\text{M}$ AngII for 3 h, which was the optimal experimental time in this study. Nuclei were then extracted and stained for p65. Staining of p65 in the nuclei of unstimulated HUVEC did not differ significantly from isotype control, indicating a low basal activity of the cells. Basal values were not altered by incubation with ramiprilat. In our assays, AngII significantly increased p65 translocation compared to untreated control (fig. 4a, b, $p < 0.01$). p65 translocation after AngII treatment reached $74 \pm 10\%$ of LPS ($1 \mu\text{g/ml}$) stimulation. The AngII-mediated p65 translocation was blocked with losartan, and therefore clearly AT1-mediated. Pretreatment with ramiprilat ($1 \mu\text{M}$) for 20 h caused

Fig. 3. AngII-induced adhesion molecule expression (**a**) and adhesion of THP-1 to HUVEC (**b**) is reduced by ramiprilat pre-treatment. **a** HUVEC were treated with ramiprilat (1 μ M) 20 h prior to AngII stimulation. Basal and stimulated MFI varied among P-selectin, VCAM-1, and ICAM-1. To allow comparisons between P-selectin, VCAM-1, and ICAM-1 levels, basal expression was set as 100% (dashed line). Bars represent expression of P-selectin, ICAM-1 and VCAM-1 expressed as percentage of basal. * $p < 0.05$, ** $p < 0.01$ compared to respective basal control, ° $p < 0.05$, °° $p < 0.01$ compared to respective AngII stimulation, $n = 6$, results are given as mean $\pm$ SD. **b** Flow chamber assays were performed as described in the Methods section. Before AngII stimulation HUVEC were treated with ramiprilat (1 μ M). Results are given as mean $\pm$ SD, * $p < 0.01$, $n = 6$.

Fig. 4. Ramiprilat treatment significantly reduces AngII-induced p65 translocation into the nucleus. HUVEC were stimulated with AngII (1 μ M) for 3 h and when indicated pretreated with losartan (1 μ M) or ramiprilat (1 μ M). Nuclear extracts were stained for p65 and analyzed using FACS. **a** Representative histogram overlay showing basal (light gray), AngII-stimulated (black) and ramiprilat-pretreated (dark gray) p65 staining in the nucleus. **b** Ordinate gives the isotype-corrected MFI for various treatments. * $p < 0.01$, $n = 6$, values are given as mean $\pm$ SD.

a significant reduction in p65 translocation (fig. 4b). To exclude contamination of nuclear fractions with cytoplasm we stained the nuclear extracts with anti-CD54 PE after HUVEC were stimulated with 1 μ M LPS for 2 h. No difference between the isotype and the basal or stimulated CD54 expression could be detected (data not shown).

Ramiprilat Downregulates AT1 Expression on HUVEC

Next, we tested the effect of ramiprilat on the expression of AT1 and AT2 receptors. Western blot analyses of

whole cell lysates of HUVEC treated for 24 h with ramiprilat in different concentrations (1 and 10 μ M) were performed (fig. 5a). Ramiprilat markedly reduced AT1 expression, while AT2 levels remained stable. As statins are known to reduce AT1 expression [15], we used simvastatin as a positive control in our experiment. Treatment of HUVEC with 1 and 10 μ M simvastatin reduced AT1 expression to a greater extent than ramiprilat. The expression of AT2 was not significantly affected (fig. 5b).

In FACS analysis we could further elucidate that downregulation of AT1 by ramiprilat is both time- (fig.

Fig. 5. Effect of ramiprilat, captopril and simvastatin on expression of AT1 and AT2 in HUVEC. **a, b** Figures show representative Western blots of whole-cell lysate for three independent experiments. In each subfigure the densitometric analysis of the band intensities is given below. $n = 3$, $* p < 0.01$, values are expressed in percent of basal expression as mean $\pm$ SD. **a** AT1/AT2 expression in HUVEC after treatment with 1 or 10 μM ramiprilat for 24 h. **b** Expression of AT1/AT2 in HUVEC after treatment with 1 or 10 μM simvastatin for 24 h. **c** HUVEC were treated with different concentrations (1 and 10 μM) of ramiprilat over various times (2 h, 12 h, 24 h). AT1 expression was assayed by FACS after staining as described in the Method section. The basal expression depicted by the dashed line is set as 100%. **d** HUVEC were treated for 24 h with either ramiprilat or captopril at different concentrations (100 nM, 1 μM , 10 μM). Data are generated and presented as described for **c**. $n = 3$, values are given as mean $\pm$ SD. * in **c** and **d** indicates significant differences compared to basal expression at $p < 0.01$.

5c) and dose-dependent (fig. 5d). While treatment of HUVEC with ramiprilat over 2 h had no significant effect on AT1 expression, prolonged treatment over 12 or 24 h caused a significant reduction in AT1 levels. After a 24-hour treatment as little as 100 nM of ramiprilat reduced AT1 expression significantly. Furthermore, an acute treatment with 10 μM ramiprilat over 15 min and subsequent fixation, staining and analysis revealed no decreased expression of AT1 (data not shown). On the other hand, treatment with captopril, another ACE inhibitor, required higher doses to influence AT1 expression significantly. 10 μM of captopril were found to be as potent as 1 μM of ramiprilat with regard to AT1 reduction (fig. 5d).

HOE-140 Partially Reverses the Ramiprilat-Mediated AT1 Downregulation and the Ramiprilat-Dependent Reduction of ICAM-1 Expression

Among the mechanisms contributing to downregulation of AT1 by ramiprilat, elevated bradykinin levels during ACE inhibition may be involved. Therefore, we tested the effect of the bradykinin 1 receptor (B1R) antagonist des-Arg⁹, [Leu⁸]-bradykinin and the bradykinin 2 receptor (B2R) antagonist HOE-140 in our system. Treatment with HOE-140 partially reversed downregulation of AT1 by ramiprilat, while incubation with the B1R antagonist had no substantial effect (fig. 6a). Combination of the two antagonists had no additional effect compared to HOE-140 alone. However, treatment with HOE-140

Fig. 6. Effect of bradykinin receptor antagonists on the ramiprilat-induced downregulation of AT1 (a) and ICAM-1 expression (b). **a** Expression of AT1 on HUVEC was analyzed by FACS. Cells were treated with ramiprilat (10 μ M, 24 h), HOE-140 (500 nM, 24 h), des-Arg⁹, [Leu⁸]-bradykinin (10 μ M, 24 h), and the combination of both antagonists combined with ramiprilat. The figure gives the relative AT1 expression compared to untreated control which is set to 100%. n = 3, values are given as mean $\pm$ SD. **b** HUVEC were treated with AngII (1 μ M, 6 h) and ramiprilat (1 μ M, 20 h prior to AngII stimulation). Where indicated, HOE-140 (500 nM) or des-Arg⁹, [Leu⁸]-bradykinin (10 μ M) were added together with ramiprilat. ICAM-1 expression was measured using FACS. Dashed line represents the basal expression of ICAM-1. * $p < 0.01$, n = 4, values are given as mean $\pm$ SD.

does not completely reverse the ramiprilat-mediated attenuation of AT1 expression, suggesting that other mechanisms may be involved in this process. Similarly, treatment with both HOE-140 and ramiprilat reduced the ramiprilat-mediated downregulation of adhesion molecules after AngII stimulation as shown for ICAM-1 in figure 6b. The B1R antagonist caused no significant effect in these experiments.

Discussion

Inflammation is an important factor contributing to the development and progression of atherosclerosis. AT1-mediated effects have been shown to be involved in various inflammatory processes which may be crucial for the onset of ischemic heart diseases or stroke [3]. In this study we describe a mechanism by which ACE inhibition reduces AT1 expression in endothelial cells, leading to decreased adhesion molecule expression and inhibition of monocyte adhesion.

In accordance with previous reports [16, 17], our study demonstrates the enhanced expression of the adhesion molecules P-selectin, VCAM-1 and ICAM-1 in response to AngII in HUVEC. These molecules have been shown to play critical roles in the recruitment of monocytes to atherosclerotic lesions [18]. Functionally, the AngII-induced expression of these adhesion molecules leads to an

increase of THP-1 cell adhesion, as observed under flow conditions. The effects of AngII on adhesion molecule expression and adhesion of THP-1 cells are clearly mediated by AT1. Although increased expression of E-selectin in response to AngII treatment has been demonstrated in previous studies [19] we were unable to confirm this finding in our experimental setting. However, differences in E-selectin expression between coronary macrovascular and microvascular endothelial cells in response to AngII have been reported [20]. Therefore, the observed lack of E-selectin induction might be attributed to the different type of endothelial cells used in this study.

Moreover, we report here that the ACE inhibitor ramiprilat reduces adhesion molecule expression in HUVEC and monocyte adhesion to these cells stimulated with AngII. Conclusively, the underlying mechanism must be independent of the suppression of AngII generation and rather interfere with RAS elements distal from AngII production. AngII receptor expression and transcription factor activity seemed to be major targets responsible for changes in adhesion molecule expression. We have previously shown that ramiprilat reduces the expression of AT1 on monocytes which may be involved in diminished chemokine expression during AngII stimulation [21]. Here we demonstrate decreased AT1 expression by ramiprilat while AT2 expression remains stable in endothelial cells. Blockage of bradykinin degradation during ACE inhibition might be of importance in this

system. Bradykinin may cause secretion of superoxide radicals and prostaglandin E_2 (PGE_2) via B2R. PGE_2 -induced cAMP elevation and enhanced superoxide radical levels destabilize AT1 mRNA with subsequent downregulation of AT1 [22–24]. To explore the role of increased bradykinin levels in the reduced expression of AT1 we used antagonists to B1R and B2R. HOE-140 clearly attenuated the ramiprilat-mediated reduction of AT1 expression and the lowering of ICAM-1 expression. However, since HOE-140 does not completely reverse the effect of ramiprilat in lowering AT1 additional mechanisms may be of importance. Hence elevated levels of NO and angiotensin (1-7) might contribute to the reduction of AT1 expression. The NO donor S-nitroso-N-acetylpenicillamine has been shown to downregulate AT1 RNA in VSMC by 90% and protein by 60% [25, 26]. Furthermore, ACE inhibitors were found to directly bind to B1R with a subsequent release of NO from endothelial cells [27]. This mechanism, however, seems less important in our system, since the B1R antagonist had no effect. Angiotensin (1-7), a degradation product of angiotensin I, has also been shown to downregulate AT1 expression [28]. Further work is required to elucidate the contribution of each mechanism to the decreased AT1 expression on monocytes and endothelial cells.

As an additional mechanism for decreased adhesion molecule expression we investigated the influence of ramiprilat on NF- κ B activity expressed as p65 translocation into the nucleus. In vivo and in vitro studies have shown the inhibition of the redox-sensitive transcription factor NF- κ B by ACE inhibitors. AngII is known to enhance NF- κ B activity in various cell types, among them monocytes [29] and endothelial cells [17, 30]. As ICAM-1, VCAM-1 and P-selectin have an NF- κ B binding site in their promoter, the enhanced expression we observed might, at least in part, be due to NF- κ B activation. The mechanism by which ACE inhibitors reduce NF- κ B activation is not known. Although most studies having dealt with this issue proclaim reduced AngII formation as main mechanism, this does not seem to be the case in our investigation. A recent study, however, reported the involvement of peroxisome proliferator-activated receptor (PPAR) α in an in vivo model of Ang-II-induced inflammation [31]. In this study, enalapril reduced the Ang-II-mediated chemokine and adhesion molecule expression in mice aortas. PPAR- α and - γ were simultaneously increased after ACE inhibitor treatment. PPARs are known to inhibit NF- κ B activation by interfering with I κ B [32, 33]. However, a plausible mechanism for the reduced p65 translocation in this study is the reduction of

AT1 expression, which may be responsible for reduced signalling events after AngII treatment. The fact that the LPS-induced CAM expression is attenuated by ramiprilat, points to the possibility that there may be AT1-independent mechanisms involved. In addition, recent publications indicate that NF- κ B is also activated after binding of AngII to AT2. However, this seems unlikely in our system, since the p65 translocation is blocked by losartan but not by PD123.319.

Accumulation of monocytes in the subendothelial space of the vessel wall is one of the earliest responses in the development of atherosclerotic lesions. Upregulation of CAMs and chemoattractants are key events for monocyte recruitment, both of which are known to be enhanced through local AngII formation. Downregulation of chemoattractant production [21] and reduced adhesion molecule expression, as found in this study and by da Cunha et al. [31], by ACE inhibitors despite AngII generation may offer an explanation for the anti-atherogenic and anti-inflammatory properties of these drugs.

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