Combined Angiotensin II Type 1 and Type 2 Receptor Blockade on Vascular Remodeling and Matrix Metalloproteinases in Resistance Arteries

Pascal Brassard, Farhad Amiri, Ernesto L. Schiffrin

Abstract—We investigated the role of angiotensin II type 1 (AT1) and AT2 receptors, matrix metalloproteinases (MMPs), and extracellular matrix (ECM) components involved in vascular remodeling of resistance arteries induced by angiotensin II (Ang II). Sprague-Dawley rats received Ang II (120 ng/kg per minute SC), the AT1 antagonist losartan (10 mg/kg per day PO), the AT1/AT2 antagonist Sar1-Ile8-Ang II (Sar-Ile; 10 μg/kg per minute SC), or hydralazine (25 mg/kg per day PO) for 7 days. Structure and mechanical properties of small mesenteric arteries were evaluated on a pressurized myograph. Ang II increased growth index (+21%), which was partially decreased by losartan (+11%) and abrogated by Sar-Ile. Hydralazine markedly increased growth index (+32%) despite systolic blood pressure (BP) lowering, suggesting a BP-independent effect of Ang II on vascular growth. Elastic modulus was increased by Sar-Ile compared with Ang II and control. Vascular type I collagen was reduced (P < 0.05), whereas fibronectin increased significantly with Sar-Ile. Vascular tissue inhibitor of metalloproteinase-2 binding to MMP-2 was abrogated by Sar-Ile, but MMP-2 activity was significantly increased compared with losartan, Ang II, and controls. Thus, AT1 blockade exerted antigrowth effects and reduced stiffness of small resistance arteries by decreasing nonelastic fibrillar components (collagen and fibronectin). Concomitant AT1/AT2 blockade prevented growth, reduced collagen type I and elastin deposition but increased vascular stiffness, fibronectin, and MMP-2 activity. These results demonstrate opposing roles of AT1 receptors that increase fibronectin and vascular stiffness and AT2 receptors that decrease MMP-2 and increase elastin. Changes in vascular wall mechanics, ECM deposition, and MMP activity are thus modulated differentially by Ang II receptors. (Hypertension. 2005;46:598-606.)

Key Words: collagen ■ extracellular matrix ■ renin-angiotensin system

Angiotensin II (Ang II), a potent vasoconstrictor of small resistance arteries, induces hypertrophy and hyperplasia of vascular smooth muscle cells (SMCs) mainly via Ang II type 1 (AT1) receptors and has been implicated in the development and maintenance of hypertension.1 Most of the biological effects of Ang II are mediated through AT1 receptors. AT2 receptor function is less well defined. Despite low AT2 receptor expression in adult tissues, their abundance is increased after blood vessel injury2 and can equal or exceed that of AT1 in pathophysiological conditions. The functional significance of AT2 receptors remains uncertain. However, a recent study demonstrated that intrabrachial infusion of the AT2 receptor antagonist PD123319 had significant systemic effects on mean arterial pressure during placebo and therapy with the AT1 receptor antagonist telmisartan without affecting forearm blood flow, suggesting the presence of functionally effective AT2 receptors. Additionally, the AT1 receptor antagonist candesartan unmasked the vasodilatory response to Ang II, suggesting that AT2 receptor blockade is responsible for increased forearm vascular resistance. In experimental models of hypertension, AT1 receptor antagonism with losartan decreased systolic blood pressure (BP) also, potentially through stimulation of AT2 receptors, the latter by increased circulating Ang II.6 Vascular AT2 receptors stimulate vasodilator cascades, including bradykinin and NO.1 Although the growth-promoting effects of Ang II appear to be mediated primarily via AT1 receptors,1 antiproliferative effects have been attributed to AT2 receptors.7 AT2 receptors have also been implicated in pathological conditions associated with cardiovascular remodeling such as neointimal formation, diabetes, and hypertension.6,8 Thus, the participation of Ang II in vascular responses after injury appears to involve AT1 and AT2 receptors.1 Vascular fibrosis involves changes in extracellular matrix (ECM) components, which include structural (collagen, elastin) and adhesion proteins (laminin, fibronectin). Collagen has been reported to be increased in resistance arteries of spontaneously hypertensive rats (SHR) and of essential hypertensive patients.9 Ang II, endothelin-1, and mineralocor-

Received May 10, 2005; first decision May 31, 2005; revision accepted June 27, 2005.
From the Canadian Institutes of Health Research, Multidisciplinary Research Group on Hypertension, Clinical Research Institute of Montreal, Quebec, Canada.
Correspondence to Ernesto L. Schiffrin, MD, PhD, FRCPC, Clinical Research Institute of Montreal, 110 Pine Ave W, Montreal, Quebec, Canada H2W 1R7. E-mail ernesto.schiffrin@ircm.qc.ca
© 2005 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org
DOI: 10.1161/01.HYP.0000176744.15592.7d

598
ticoids appear to play a central role in this process. Indeed, Ang II stimulates human vascular SMC (VSMC) production of collagen I and fibronectin via AT1 receptors and may do so as well via AT2 receptors.10 Vascular remodeling entails degradation and reorganization of the ECM scaffolding, which may explain the recent interest in matrix metalloproteinases (MMPs) participating in these processes. Several MMPs in the vasculature, such as collagenases and gelatinases, have been implicated in atherosclerosis, restenosis, and hypertension.11 Changes in MMP activity or in the balance with their natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs), may contribute to resistance artery remodeling by modulating the composition of ECM.

We hypothesized that vascular remodeling in Ang II–induced hypertension can be mediated by AT1 and AT2 receptors by altering MMP and TIMP activity, which could affect ECM content and vascular mechanics. Therefore, we evaluated the effects of AT1 and AT2 receptor blockade on vascular mechanics, ECM components, MMPs, and TIMPs involved in vascular remodeling of resistance arteries of Ang II–infused rats.

Methods

Animal Experiments

The study protocol was approved by the animal care committee of the Clinical Research Institute of Montreal and performed following recommendations of the Canadian Council of Animal Care. Male Sprague-Dawley rats (Charles River; St. Constant, Quebec, Canada; n = 8 per group) were housed under controlled conditions and infused with Ile5-Ang II (120 ng/kg per minute; Calbiochem) via osmotic minipumps (Alzet Corp.). Losartan (10 mg/kg per day) and hydralazine (25 mg/kg per day) were added in the drinking water, whereas Sar1-Ile8-Ang II (Sar-Ile; an AT1/AT2 antagonist; Bachem) was infused via osmotic mini-pumps (10 µg/kg per minute), all administered for 7 days. Before the end of study, systolic BP (SBP) was measured by the tail-cuff method as described previously,12 and rats were then killed humanely.

Preparation and Mounting of Small Arteries

Third-order branches of superior mesenteric arteries were dissected and placed in cold physiological salt solution (PSS) containing (in mmol/L): 120 NaCl, 25 NaHCO3, 4.7 KCl, 1.18 KH2PO4, 1.2 MgSO4, 2.5 CaCl2, 0.026 EDTA, and 5.5 glucose. Vessels were mounted on a pressurized myograph and equilibrated for 1 hour in PSS containing 10 mmol/L EGTA (bubbled with 95% air–5% CO2, pH 7.4) at 37°C. Only vessels that responded with >70% vasoconstriction to extraluminal application of 0.9% KC1+10 µmol/L norepinephrine were used. Pressure–lumen diameter relationship was assessed as intravascular pressure was increased step-wise from 3 to 140 mm Hg and media thickness and lumen diameter were measured at each pressure level.12

Vascular Measurements and Mechanics

Media cross-sectional area (CSA) was obtained by subtraction of internal CSA from external CSA using internal and lumen diameters.12 Growth index was calculated as (CSA/vessel area)−(CSA/vessel area), where CSA and CSA0 are media CSA of normotensive and hypertensive vessels, respectively. Additionally, mechanical properties of blood vessels (circumferential strain, circumferential stress, and incremental elastic modulus) were calculated as described previously.13

Western Blot Analysis of Vascular AT1 and AT2 Receptors

Protein was extracted from frozen aortas by homogenization, and Western analysis of AT1 and AT2 receptors were done as described previously.15

Collagen and Elastin Determination

Pressurized mesenteric arteries fixed in Russell fixative were embedded in paraffin while aortas were frozen in optimal cutting temperature compound and used for collagen and elastin histochemistry. For collagen type I quantification, 10-µm sections were stained with Sirius red F,B,A (0.1% pH 2.0 in saturated picric acid solution; Sigma) and analyzed by cross-polarized microscopy as described previously.14 Images were analyzed by Northern Eclipse 5.0 (EM-Pix Imaging Inc). Elastin was stained with Verhoef van Giesson, whereas fibronectin immunohistochemistry (Chemicon) was performed on paraffin-embedded sections and examined under light microscopy. Sections were counterstained with hematoxylin while negative controls were incubated with nonimmune rabbit IgG in place of primary antibody.

Zymography and Reverse Zymography

Protein was extracted from frozen aortas by homogenization. Briefly, homogenates were resuspended in lysis buffer (0.05 mol/L Hepes, pH 7.4, 0.15 NaCl mol/L, 1% Nonidet P-40 (Roche Molecular Biochemicals), 1 mmol MgCl2/L, 1 mmol/L CaCl2, and 1 tablet of Complete mini EDTA-free Protease Inhibitor Cocktail (Roche Molecular Biochemicals), centrifuged (15 000g), and protein concentration was determined with the Bio-Rad Dc protein assay. Latent and activated gelatinases were detected with SDS-PAGE gelatin zymography. After gel staining, MMP-2 and MMP-9 were identified on the basis of gelatin lysis at 62 and 82 kDa for activated MMP-2 and MMP-9, respectively. Gelatinolytic bands were quantified using ImageQuant software 5.0 (Molecular Dynamics). Reverse zymography was performed as described by Oliver et al. Briefly, gelatinase inhibitory activity was detected by incubating standard gelatin zymograms in which purified gelatina A (0.16 µg/mL) was added. Gels were incubated with 2.5% Triton X-100 and thereafter at 37°C for 18 hours in an activity buffer (50 mmol/L Tris-HCl, pH 8, containing 5 mmol/L CaCl2, 0.2 mol/L NaCl, 0.02% NaN3, and 0.02% wt/vol BRIJ-35). Gels were stained and destained as described above. Stained bands represented gelatinase inhibitory activity corresponding to TIMP binding and thus inhibiting MMP-2 activity.16

Statistical Analysis

Data are presented as mean±SEM. Morphological data and band optical density were analyzed by 1-way ANOVA followed by a Student Newman–Keuls test, whereas mechanical parameters were compared by repeated-measures 1-way ANOVA. A value of P<0.05 was considered statistically significant.

Results

Physiological Characteristics

SBP was significantly increased (P<0.001) by Ang II infusion compared with controls (Table 1). This increase was significantly blunted by losartan, hydralazine, and Sar-Ile. Body weight did not differ among groups. Ang II and Sar-Ile resulted in increased heart weight relative to tibia length compared with controls, whereas losartan and hydralazine prevented this increase (Table 1). Aortic CSA and media-to-lumen (M/L) ratio were similar in all groups (Table 1).

Vascular Mechanics

Lumen diameter of small arteries measured at increasing intraluminal pressures was significantly smaller in all treated groups compared with controls (P<0.001; Figure 1A). Hydralazine and Sar-Ile significantly decreased lumen diameter when compared with Ang II (P<0.05 and P<0.001, respectively; Figure 1A) whereas media thickness was significantly decreased by losartan, hydralazine, and Sar-Ile (P<0.001 versus controls and Ang II; Figure 1B). M/L ratio was similar in all groups except after losartan, which significantly de-
creased it ($P<0.05$ versus Ang II; Figure 1C). Ang II increased the growth index ($+21\%$), which was decreased by losartan ($-11\%$) and Sar-Ile ($-1\%$) but further increased by hydralazine ($+32\%$).

Resistance arteries from Ang II–treated rats showed reduced strain ($P<0.001$ versus controls), which was partially restored by losartan but significantly decreased by Sar-Ile (Figure 1D). The leftward shift of the stress–strain curve in vessels from Ang II–treated rats suggested vessel stiffening, which was partially decreased by losartan but increased by hydralazine and Sar-Ile (Figure 1D). However, incremental elastic modulus was unaffected by Ang II and losartan. In contrast, elastic modulus was greater in hydralazine and in Sar-Ile compared with controls ($P<0.001$). Incremental elastic modulus as a function of media stress, which evaluates the stiffness of wall components independently of vessel geometry, was similar in all groups (Figure 1E).

**Western Blot Analysis of Vascular AT$_1$ and AT$_2$ Receptors**

Ang II had a tendency to decrease vascular AT$_1$ receptor expression, whereas receptor expression increased significantly after AT$_1$ antagonism by losartan ($P<0.05$; Figure 2). No changes were observed with Sar-Ile treatment, whereas

---

**TABLE 1. Physiological and Vascular Morphological Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ang II</th>
<th>Ang II + Los</th>
<th>Ang II + Sar-Ile</th>
<th>Ang II + Hyd</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>113±2</td>
<td>177±7*</td>
<td>139±4†‡</td>
<td>154±7*‡</td>
<td>134±7§</td>
</tr>
<tr>
<td>HW/TL (g/mm)</td>
<td>31.1±0.3</td>
<td>35.1±1.2*</td>
<td>32.0±1.1†‡</td>
<td>36.7±1.4*</td>
<td>32.9±1.4†‡</td>
</tr>
<tr>
<td>Aorta CSA ($\times 10^{4}$ µm$^2$)</td>
<td>1.6±0.2</td>
<td>1.5±0.3</td>
<td>1.6±0.2</td>
<td>2.1±0.1</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>Aorta M/L ratio (%)</td>
<td>8.5±0.3</td>
<td>8.4±1.0</td>
<td>8.8±0.7</td>
<td>10.7±0.9</td>
<td>9.0±0.9</td>
</tr>
</tbody>
</table>

* $P<0.001$ vs control; † $P<0.01$ vs control; ‡ $P<0.01$ vs Ang II; § $P<0.05$ vs control; †† $P<0.001$ vs Ang II.

HW indicates heart weight; TL, tibia length.
hydralazine significantly reduced AT1 receptor expression (P<0.05 versus losartan; Figure 2). No changes were observed in AT2 receptor expression among the groups (data not shown).

**Vascular Wall ECM Content**

Mesenteric arteries of Ang II–infused rats exhibited a trend to increase type I collagen compared with controls, whereas Sar-Ile significantly decreased it (P<0.05 versus losartan; Table 2). Ang II reduced elastin content compared with controls (P<0.01), a change prevented by losartan (P<0.05 versus Ang II) and unaffected by Sar-Ile (P<0.001 versus control; P<0.01 versus losartan). Hydralazine induced a trend to a reduction in elastin to similar levels as in the Ang II group, demonstrating that this effect was independent of BP. The collagen-to-elastin ratio was increased significantly by Ang II compared with other groups, whereas a marked increase in fibronectin content was found in the Sar-Ile–treated group compared with other groups (Figure 3). In aorta, type I collagen was significantly increased by Ang II (P<0.05 versus control), significantly lowered by losartan (P<0.01 versus Ang II) and Sar-Ile (P<0.05 versus Ang II), and unaltered by hydralazine, suggesting an AT1-dependent, BP-independent effect. Similarly, fibronectin was increased by Ang II (P<0.05 versus control) and significantly reduced by losartan (P<0.05 versus Ang II) but not by Sar-Ile (P<0.001 versus control; P<0.01 versus losartan; Table 2).

**Gelatinase Activity**

Gelatin zymographic analysis revealed several bands representing lytic activity in aorta. Sar-Ile induced a significant increase in 62 kDa lytic band, which corresponds to the active form of constitutive MMP-2 compared with controls (Figure 4). The active form of MMP-9, which is an inducible MMP, was undetectable. Nevertheless, there was a significant increase in all other forms of MMP-9 with Sar-Ile, including dimerized MMP-9 (data not shown). All lytic bands were inhibited in presence of EDTA (data not shown). Ang II significantly increased TIMP-2 expression in aorta (P<0.05 versus control); whereas losartan significantly reduced TIMP-2 expression (P<0.05 versus Ang II). Sar-Ile and hydralazine increased it to a greater extent than losartan compared with controls (data not shown). In mesenteric arteries, TIMP-2 increase was induced only by Ang II, whereas expression levels remained very low in all other groups (Figure 5). MMP inhibitor activity by reverse zymography was only investigated on aorta because of limited mesenteric vessel availability. No significant differences were found in TIMP-1 levels between groups because of high variability, although a trend to increase in TIMP-1 binding to MMP-2 was observed with Ang II. Sar-Ile abrogated TIMP-2 binding to MMP-2 (P<0.01 versus Ang II; Figure 6), whereas it significantly stimulated MMP-2 activity as determined by MMP-2/TIMP-2 ratio (P<0.001; Figure 7).

**Discussion**

It has been proposed that AT1 and AT2 receptors have countervailing effects on BP regulation. Thus, it might be expected that simultaneous blockade of AT1 and AT2 receptors would result in reduced effects relative to selective blockade of AT1 receptors. The present study demonstrates that concomitant blockade of AT1 and AT2 by Sar-Ile altered ECM deposition and mechanical properties of mesenteric resistance arteries, which participate in the vascular structural changes occurring after Ang II infusion, differentially from selective blockade of AT1 receptors. Ang II–induced alterations in mechanical properties of mesenteric resistance arteries are determined, at least in part, by changes in the

**TABLE 2. Relative Amounts of Media ECM Components in Percent**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ang II</th>
<th>Ang II + Los</th>
<th>Ang II + Sar-Ile</th>
<th>Ang II + Hyd</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aorta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen type I</td>
<td>12.1±3.7</td>
<td>25.9±4.2*</td>
<td>10.9±2.4†</td>
<td>14.2±3.6‡</td>
<td>23.8±4.2$§</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>3.1±1.8</td>
<td>18.7±3.5*</td>
<td>6.5±3.2‡</td>
<td>27.7±5.8¶</td>
<td>21.6±4.9#¶</td>
</tr>
<tr>
<td><strong>Mesenteric arteries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen type I</td>
<td>41.2±7.8</td>
<td>51.9±1.5</td>
<td>38.4±4.2</td>
<td>12.8±5.1†</td>
<td>29.7±6.8</td>
</tr>
<tr>
<td>Elastin</td>
<td>63.9±3.2</td>
<td>44.1±3.6#</td>
<td>56.4±2.0‡</td>
<td>38.6±2.8¶</td>
<td>44.3±7.4</td>
</tr>
<tr>
<td>Collagen/elastin</td>
<td>6.4±1.1</td>
<td>12.0±1.3*</td>
<td>6.8±7.8‡</td>
<td>3.5±1.7</td>
<td>7.0±1.5‡</td>
</tr>
</tbody>
</table>

*P<0.05 vs control; †P<0.01 vs Ang II; ‡P<0.05 vs Ang II; §P<0.05 vs Ang II + Los; ¶P<0.001 vs control; ††P<0.01 vs Ang II + Los; ‡‡P<0.01 vs control.

Values are expressed in relative intensity/area (pixels).
structure and the composition of the vascular wall that may be attenuated by AT1 antagonism, independently of BP reduction. The present study suggests a role of AT2 receptors in the regulation of ECM deposition in the media of resistance arteries after Ang II infusion because blockade of AT2 receptors leads to increased stiffness of the vascular wall.

Vascular remodeling in hypertension involves 2 processes: (1) media remodeling through growth, regression, contraction, or relaxation and apoptosis of VSMCs, and (2) ECM expansion or contraction. Growth of resistance artery wall induced by Ang II (+11%) was partially reduced by losartan (-11%), confirming previous findings that Ang II–induced vascular growth is mediated by the AT1 receptor in aorta and mesenteric arteries. Growth index was enhanced by hydralazine (+32%), which, in addition to its vasodilator properties, has been suggested to have trophic effects mediated by hypoxia-inducible factor-1α induction of mitogen-activated protein kinase pathways. Sar-Ile abrogated growth (-1%), which can be explained mainly by blockade of the AT1 receptor that exerts growth-promoting effects and inhibition of the AT2 antiproliferative effects. Most studies, conducted in normotensive rats, have demonstrated that AT2 stimulation was associated with vasodilation and inhibition of cell growth. Previous in vivo studies have revealed controversial results concerning the role of AT2 on VSMC growth in different rat models of experimental hypertension. Although it has been reported that the AT2 receptor mediated aortic and coronary artery hypertrophy and differentiation, we reported that AT1 induced vascular hypertrophy in different vascular beds in chronic Ang II–infused Wistar rats, and that this remodeling was unaffected by AT2 blockade. However, Cao et al demonstrated that AT1 and AT2 receptors mediated mesenteric vascular hypertrophy and VSMC proliferation. It has also been suggested that AT2 receptors may contribute to aortic SMC hypertrophy in SHR. Thus, absence of stimulation of both Ang II receptor subtypes by blockade by Sar-Ile may explain the robust inhibition of growth found in the present study. The exact role of AT2 receptors in the regulation of VSMC growth and differentiation in hypertension evidently requires further clarification. One-week Ang II infusion caused eutrophic remodeling of resistance arteries, as demonstrated by increased M/L ratio, with no

![Figure 3. Immunohistochemical staining of fibronectin in mesenteric arteries. Representative images of fibronectin immunostaining in control (A), Ang II (B), losartan (C), Sar-Ile (D), or hydralazine (E). F, Negative control. Brown represents fibronectin staining; nuclei appear blue.](image)

![Figure 4. MMP-2 activity in aorta. Top, Representative gel of a gelatin zymogram of MMP-2 activity. Bottom, Results are means ± SEM. *P < 0.05 vs control. Los indicates losartan; Hyd, hydralazine.](image)
change in CSA as we had previously reported. In other types of experimental models of hypertension such as SHR, eutrophic or hypertrophic remodeling may be found in different experiments. Aortas from Sar-Ile–treated rats exhibited eutrophic remodeling, as shown by reduced lumen diameter, with no change in net growth and media CSA. As for mesenteric resistance vessels, we found inward eutrophic remodeling, as evidenced by decreased lumen diameter and media thickness, with no change in media CSA. This remodeling may be the result of rearrangement of cellular and noncellular components, combination of growth and apoptosis, or a persistent contracted state of SMCs with a resultant absence of change in the total amount of wall material.

Associated with structural changes, significant alteration in resistance artery wall mechanics may be found. One of the 2 novel findings of this study was that the vessel wall of the Sar-Ile group was stiffer, as demonstrated by the leftward shift of the stress–strain relationship (Figure 1D). Incremental elastic modulus, which evaluates stiffness of wall components independently of vessel geometry, was increased by Ang II, exacerbated by hydralazine, and increased to a greater extent by simultaneous blockade of AT_1 and AT_2 receptors by Sar-Ile (Figure 1E). Increased stiffness was prevented by losartan, suggesting that this effect was mediated by AT_1 receptors. The paradoxical increase in vascular stiffness after Sar-Ile, together with the losartan data, suggests that this effect was the result of blockade of AT_2 receptors. This indicates that AT_2 receptors have a beneficial effect on vascular stiffness. Increased wall stiffness was mediated by BP-independent mechanisms in Ang II–infused rats, as shown by the greater stiffness found after combined blockade of both Ang II receptor subtypes with Sar-Ile and under hydralazine treatment.

We observed a tendency of decreasing AT_1 receptor expression after Ang II infusion as shown previously. AT_1
stiffness. After 1 week of Ang II infusion, significant changes occur after AT2 receptor blockade, which support our findings.4,5,42 We found a significant increase in SBP when AT1 and AT2 receptors were blocked, suggesting that the BP-lowering effect of losartan can be mediated in part via unblocked AT1 receptors stimulated by the increased circulating Ang II present in this experimental paradigm. Moreover, Barber et al43 also demonstrated that the AT2 receptor blockade offset the effect of candesartan, whereas its stimulation with CGP42112 reinforced the BP-lowering effect of the AT1 antagonist.6

The ECM organization under normal and pathological conditions results from a balance between synthesis and degradation of extracellular proteins, a process in which MMPs play a major role.39 The majority of MMPs are normally expressed at low levels, but their expression is stimulated in hypertension.40 Significant increases in MMP-2 activity were found with Sar-Ile, whereas TIMP-2 binding activity was inhibited (Figures 4 and 6, respectively). This explains in part the decrease in vascular type I collagen and elastin and subsequent decrease in the collagen/elastin ratio. These changes in ECM can be associated with modulation of cell–fibrillar ECM attachment sites and potentially influence the effect of ECM protein deposition on vascular compliance.32 We demonstrated that regulation of ECM deposition was mediated partly by AT2 receptors because blockade of this receptor (together with AT1) by Sar-Ile reduced TIMP-2 binding (Figure 6). Contrary to our results, Rizzoni et al demonstrated that subcutaneous arteries from hypertensive patients treated with either the AT1 antagonist candesartan or the ACE inhibitor enalapril had decreased MMP-9 activity and collagen content with candesartan treatment only.41 These findings suggest a role of AT1 receptor blockade in modulation of collagen and MMP activity in human resistance vessels, whereas we found no increase in MMP-9 activity in the losartan-treated group.

Losartan and hydralazine reduced the Ang II–induced BP rise. However, Sar-Ile–treated animals had higher SBP than losartan but significantly lower SBP than Ang II, which could be explained by the absence of beneficial effects mediated by AT2. A very limited number of studies have been done in humans in whom it has been demonstrated that hemodynamic changes occur after AT2 blockade, which support our findings.4,5,42 We found a significant increase in SBP when AT1 and AT2 receptors were blocked, suggesting that the BP-lowering effect of losartan can be mediated in part via unblocked AT1 receptors stimulated by the increased circulating Ang II present in this experimental paradigm. Moreover, Barber et al43 also demonstrated that the AT2 receptor blockade offset the effect of candesartan, whereas its stimulation with CGP42112 reinforced the BP-lowering effect of the angiotensin receptor blocker. Finally, Gigante et al found that in salt-restricted rats, the AT2 blocker PD123319 offset the BP-lowering effect of losartan, suggesting that AT2 receptors contribute to the hypotensive effects of the AT1 antagonist.6

There are limitations to this study. The use of a selective AT1 receptor antagonist alone and in combination to losartan would have been informative, but because of limited availability and prohibitive high cost, this could not be performed and was replaced by the use of Sar-Ile. MMP activity known to be vasorelaxant and antiproliferative through NO production.36 However, during AT1 receptor blockade, no increase in bradykinin occurs because its degradation by angiotensin-converting enzyme (ACE) is not inhibited. However, because AT2 stimulation has been shown to increase bradykinin locally at the cellular level,32 this could potentially explain the deleterious effects of AT1 receptor blockade, as is the case when we administered Sar-Ile, as suggested by Aartsen et al.38

The ECM organization under normal and pathological conditions results from a balance between synthesis and degradation of extracellular proteins, a process in which MMPs play a major role.39 The majority of MMPs are normally expressed at low levels, but their expression is stimulated in hypertension.40 Significant increases in MMP-2 activity were found with Sar-Ile, whereas TIMP-2 binding activity was inhibited (Figures 4 and 6, respectively). This explains in part the decrease in vascular type I collagen and elastin and subsequent decrease in the collagen/elastin ratio. These changes in ECM can be associated with modulation of cell–fibrillar ECM attachment sites and potentially influence the effect of ECM protein deposition on vascular compliance.32 We demonstrated that regulation of ECM deposition was mediated partly by AT2 receptors because blockade of this receptor (together with AT1) by Sar-Ile reduced TIMP-2 binding (Figure 6). Contrary to our results, Rizzoni et al demonstrated that subcutaneous arteries from hypertensive patients treated with either the AT1 antagonist candesartan or the ACE inhibitor enalapril had decreased MMP-9 activity and collagen content with candesartan treatment only.41 These findings suggest a role of AT1 receptor blockade in modulation of collagen and MMP activity in human resistance vessels, whereas we found no increase in MMP-9 activity in the losartan-treated group.

Losartan and hydralazine reduced the Ang II–induced BP rise. However, Sar-Ile–treated animals had higher SBP than losartan but significantly lower SBP than Ang II, which could be explained by the absence of beneficial effects mediated by AT2. A very limited number of studies have been done in humans in whom it has been demonstrated that hemodynamic changes occur after AT2 blockade, which support our findings.4,5,42 We found a significant increase in SBP when AT1 and AT2 receptors were blocked, suggesting that the BP-lowering effect of losartan can be mediated in part via unblocked AT1 receptors stimulated by the increased circulating Ang II present in this experimental paradigm. Moreover, Barber et al43 also demonstrated that the AT2 receptor blockade offset the effect of candesartan, whereas its stimulation with CGP42112 reinforced the BP-lowering effect of the angiotensin receptor blocker. Finally, Gigante et al found that in salt-restricted rats, the AT2 blocker PD123319 offset the BP-lowering effect of losartan, suggesting that AT2 receptors contribute to the hypotensive effects of the AT1 antagonist.6

There are limitations to this study. The use of a selective AT1 receptor antagonist alone and in combination to losartan would have been informative, but because of limited availability and prohibitive high cost, this could not be performed and was replaced by the use of Sar-Ile. MMP activity known to be vasorelaxant and antiproliferative through NO production.36 However, during AT1 receptor blockade, no increase in bradykinin occurs because its degradation by angiotensin-converting enzyme (ACE) is not inhibited. However, because AT2 stimulation has been shown to increase bradykinin locally at the cellular level,32 this could potentially explain the deleterious effects of AT1 receptor blockade, as is the case when we administered Sar-Ile, as suggested by Aartsen et al.38
determination was only conducted on aortas and not mesenteric arteries because of limited mesenteric artery availability. Mesenteric arteries are representative of resistance vessels and our data from aortic tissue cannot be directly extrapolated to other segments of the vasculature.

In conclusion, the present study showed that blockade of AT1 and AT2 receptors in Ang II–infused rats was associated with severe changes in structure, mechanics, and composition of small mesenteric resistance arteries. In contrast, Ang II–induced changes were prevented in large part by the AT2 receptor blocker losartan and were independent of BP reduction. These results underscore the vascular protective role played by AT2 receptors. Increases in stiffness in response to Ang II may be explained in part by the greater collagen/elastin ratio, although enhanced expression of fibronectin may play a more important role. Decreases in collagen and elastin content may be a consequence of upregulation of MMP-2 activity as well as a reduction in TIMP-2 binding to MMP-2, resulting in an increased activity of the protease when AT1 and AT2 receptors are blocked.

**Perspectives**

These results demonstrate the differential importance of AT1 and AT2 receptors for the maintenance of vascular structural integrity. AT1 and AT2 receptors play opposing roles on ECM deposition: AT1 receptors enhance fibronectin deposition and vascular stiffness, and AT2 receptors reduce stiffness and fibronectin and increase elastin in resistance arteries. Differential regulation of ECM and vascular stiffness by AT1 and AT2 receptors may explain in part the different effects found on cardiovascular risk reduction after treatment with ACE inhibitors and angiotensin receptor blockers in various cardiovascular conditions.

**Acknowledgments**

This work was supported by grant 13570 (E.L.S.) and a group grant to the Multidisciplinary Research Group on Hypertension from the Canadian Institutes of Health Research (CIHR). The authors are grateful to Suzanne Diébold and André Turgeon for their excellent technical assistance and to Christian Charbonneau, Dr Jean-François Brassard et al. Ang II, MMPs, and Vascular Remodeling 605

**References**


Combined Angiotensin II Type 1 and Type 2 Receptor Blockade on Vascular Remodeling and Matrix Metalloproteinases in Resistance Arteries
Pascal Brassard, Farhad Amiri and Ernesto L. Schiffrin

Hypertension. 2005;46:598-606; originally published online July 25, 2005;
doi: 10.1161/01.HYP.0000176744.15592.7d
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/46/3/598

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/