Effects of AT\(_1\) and AT\(_2\) Angiotensin Receptor Antagonists in Angiotensin II–Infused Rats

Jin S Li, Rhan M Touyz, Ernesto L Schiffrin

Abstract—Angiotensin II (Ang II) appears to exert its contractile and growth-promoting effects through the AT\(_1\) receptor subtype, whereas the AT\(_2\) subtype may have growth-inhibitory and proapoptotic properties. Recently, some data have challenged this emerging concept. To clarify the role of AT\(_1\) and AT\(_2\) receptors, we treated Wistar rats that were infused with Ang II (120 ng/kg/min subcutaneously by osmotic minipump), with the AT\(_1\) antagonist losartan (10 mg/kg/d in the drinking water) and the AT\(_2\) antagonist PD123319 (30 mg/kg/d subcutaneously by osmotic minipump) for 21 days. At the end of the study, tail-cuff systolic blood pressure was 106±2.8 mm Hg in untreated rats and 108±2.7 mm Hg in rats infused with Ang II that received losartan, whereas it rose to 158±4.3 mm Hg in Ang II-infused rats and 158±3.0 mm Hg in rats infused with Ang II and PD123319 (the two latter groups \(P<0.01\) versus the two other groups). Heart weight, and aorta cross-section/body weight ratio were higher in Ang II–infused rats than in controls and were significantly reduced in Ang II–infused rats that received losartan \(P<0.05\). Wire-myograph-mounted coronary, renal, mesenteric, and femoral small arteries from Ang II–infused rats and Ang II–infused rats receiving PD123319 had a greater media, media cross-section, and media/lumen ratio than vessels from untreated or Ang II–infused rats treated with losartan. These results support the concept that in Wistar normotensive rats infused for 3 weeks with angiotensin II, growth in the heart, aorta, and coronary, renal, mesenteric, and femoral small arteries is mediated by the AT\(_1\) receptor. The results show little evidence of a role of AT\(_2\) receptors in mediating angiotensin II effects in this experimental paradigm (Hypertension. 1998;31[part 2]:487-492.)

Key Words: blood pressure ■ small arteries ■ resistance arteries ■ remodeling ■ vascular smooth muscle cells ■ growth ■ vascular hypertrophy

Ang II, the final mediator of the renin-angiotensin system, plays a pivotal physiological role in cardiovascular homeostasis. It is a potent vasoconstrictor of the peripheral vasculature and induces growth of smooth muscle cells of blood vessels and in the heart. Because of these actions, Ang II may play an important pathophysiological role in the development and maintenance of hypertension. Cellular responses to Ang II are mediated by specific cell membrane receptors. Two main subtypes of angiotensin receptors have been pharmacologically defined and cloned: AT\(_1\) receptors, which are blocked specifically by losartan, and AT\(_2\) receptors, which are blocked specifically by PD123319. Ang II appears to exert its contractile and growth-promoting effects through the AT\(_1\) receptor subtype, whereas the AT\(_2\) subtype has been suggested to have growth-inhibitory and proapoptotic properties. Recently, some data have challenged this emerging concept. In two successive publications, investigators obtained results suggesting that when Ang II was infused subcutaneously for 3 weeks into Wistar rats, the increase in blood pressure that occurs in this experimental paradigm is mediated by AT\(_1\) receptors, as shown by abrogation of this effect by concurrent administration of losartan, an AT\(_1\) angiotensin antagonist. In contrast, medial hypertrophy of aorta and coronary arteries was abolished by concomitant administration of the AT\(_2\) receptor antagonist PD123319. The Ang II–dependent growth of vascular smooth muscle cells was associated with reversal to an immature phenotype, as shown by the expression of cellular fibronectin and nonmuscle myosin, which was abolished when losartan was coadministered with Ang II. In addition, administration of losartan alone to normal rats reproduced the Ang II–induced vascular hypertrophy. The authors concluded that Ang II–induced growth of vascular smooth muscle cells is mediated by AT\(_2\) receptors, whereas the phenotypic changes of smooth muscle cells are AT\(_1\)–dependent.

To clarify the role of AT\(_1\) and AT\(_2\) receptors in Ang II–induced growth, we have reproduced in part the experiments reported previously and have examined morphometrically small resistance size arteries in the coronary, renal, mesenteric, and femoral circulation for evidence of growth in the presence of AT\(_1\) and AT\(_2\) receptor antagonists. We treated Wistar rats that were infused with Ang II (120 ng/kg/min subcutaneously by osmotic minipump), with the AT\(_1\) antagonist losartan (10 mg/kg/d in the drinking water) and/or the AT\(_2\) antagonist PD123319 (30 mg/kg/d subcutaneously by osmotic minipump) for 21 days. At the end of the study,
cil, renal, mesenteric, and femoral small arteries were investigated on a wire-myograph. Our results support the concept that in Wistar normotensive rats infused for 3 weeks with Ang II, growth in the heart, aorta, and coronary, renal, mesenteric, and femoral small arteries is mediated by the AT$_1$ receptor, our results show little evidence of a role of AT$_2$ receptors in mediating Ang II effects in this experimental paradigm.

**Materials and Methods**

Animal experiments were performed according to the recommendations of the Canadian Council for Animal Care and were approved by the Animal Care Committee of the Clinical Research Institute Wistar. Rats weighing 200 g were bought from Charles River. They were housed in individual cages under conditions of constant temperature (22°C) and humidity (60%) and exposed to a 12-hour light/dark cycle. An osmotic minipump (Alzet, model 2ML4) was implanted subcutaneously in the back of the neck of each rat under anesthesia with sodium pentobarbital (Somnotol), 50 mg/kg intraperitoneally. The thoracic aorta was dissected out and immediately frozen in dry ice. Slices of 8-μm-thick sections, fixed, and stained with hematoxylin-eosin were obtained from the media thickness (m) and the circumference of blood vessels (at Lo) was then calculated. The lumen diameter was obtained by using L, and the calculated media cross-sectional area and assuming a constant media volume, the standardized media thickness of blood vessels (at Lo) was then calculated. The lumen diameter was obtained as $L_0/\pi$.

**Preparation of Small Arteries**

Coronary, renal arcuate, and femoral small arteries were obtained as we have previously described. In male rats weighing 200 g, the heart and aorta were dissected out, blotted dry, and weighed. The thoracic aorta was dissected out and immediately frozen in dry ice and kept at −70°C until used. Frozen segments of aorta were cut in 8-μm-thick sections, fixed, and stained with hematoxylin-eosin. The cross-sectional area of the intima media of the aorta was evaluated from micrographs of whole aortic ring sections taken at 10× magnification and scanned, digitized, and analyzed by computer, using Adobe Photoshop Imaging software (Adobe System Corporation). Blood pressure did not change in untreated rats (Fig 1 and Table 1). In Ang II-infused rats, systolic blood pressure rose 50 mm Hg more than in untreated rats (Table 1 and Fig 1), whereas blood pressure was unchanged in rats infused with Ang II that received losartan. Losartan alone had no effect on blood pressure Ang II-infused rats that received simultaneously an infusion of PD123319 showed a rise in blood pressure similar to that of rats receiving only Ang II.

**Results**

Body weight was similar in all rats except in Ang II-infused rats receiving losartan, in which it was lower ($P<0.05$) than that of untreated rats (Table 1). The heart/body weight ratio and the cross-sectional area of the intima media of the aorta were increased ($P<0.05$) by the Ang II infusion. In losartan-treated rats infused with Ang II, the heart/body weight ratio was smaller ($P<0.05$) and the cross-sectional area of the intima media of the aorta was lower ($P<0.05$ by Student's t-test) than in Ang II-infused rats. Interestingly, the cross-sectional area of aorta (corrected or not for body weight) of rats treated only with losartan or infused simultaneously with Ang II and with the AT$_2$ antagonist PD123319 achieved similar values, not significantly different from those of either untreated or Ang II-infused rats but intermediate between these two groups.
Wire-myograph-mounted coronary, renal, mesenteric, and femoral small arteries had a greater media width, media cross-sectional area, and media/lumen ratio in Ang II-infused rats and in Ang II-infused rats receiving PD123319 than in untreated rats or Ang II-infused rats treated with losartan (Table 2 and Fig 2, P<0.01) Losartan alone had no effect on small artery structure.

Discussion

Besides its vasoconstrictor role, Ang II acts as a growth-promoting agent. It induces growth (hypertrophy and/or hyperplasia) in many cultured cell types and in vivo. Like most of the known physiological effects of Ang II, evidence up to the present has suggested that these trophic effects are mediated by AT₁ receptors. Indeed, until recently, it was believed that vascular Ang II receptors were exclusively of the AT₁ subtype. However, the adult rat aorta expresses a small but significant amount of AT₂ receptors as well. In aorta of fetal and young rats, the proportion of AT₂ receptors is higher, this predominance of AT₂ receptors is reversed during development. In 2-week-old Sprague-Dawley rats, 81% of angiotensin receptors in the aorta are of the AT₂ subtype, in 8-week-old rats, this is reduced to 28%, with a predominance of AT₁ (71%) In 6- to 8-week-old SHR and WKY rats, renal resistance vessels display 20% of Ang II binding sites with affinity to PD123319. Recent studies have contributed to elucidate in part the elusive role of AT₂ receptors. Stoll et al demonstrated an antiproliferative effect of Ang II on coronary endothelial cells, which could be blocked by the AT₂ antagonist PD123177, and recent work has implicated AT₂ receptors in induction of apoptosis. However, controversial studies have suggested a complex interaction of angiotensin receptor subtypes in the regulation of growth. Saward et al reported that PD123319 but not losartan could block Ang II-induced RNA synthesis in A10 vascular smooth muscle cells. Finally, two recent studies in which Wistar rats were treated for 3 weeks with Ang II and PD123319 showed that blood pressure remained high but fibrosis and vascular hypertrophy were reduced by the AT₂ antagonist compared to Ang II infusion alone. In contrast to the antihypertrophic effect of the AT₂

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Ang II</th>
<th>Losartan</th>
<th>Ang II+Losartan</th>
<th>Ang II+PD123319</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>106±3</td>
<td>158±5**</td>
<td>108±2</td>
<td>108±2</td>
<td>158±3**</td>
</tr>
<tr>
<td>BW (g)</td>
<td>359±8</td>
<td>327±8*</td>
<td>340±7</td>
<td>320±3**</td>
<td>336±3</td>
</tr>
<tr>
<td>HW (g)</td>
<td>1.24±0.02</td>
<td>1.24±0.06</td>
<td>1.14±0.03</td>
<td>1.09±0.03†</td>
<td>1.25±0.03</td>
</tr>
<tr>
<td>HW (g)/100 g BW</td>
<td>0.345±0.010</td>
<td>0.377±0.010</td>
<td>0.336±0.009</td>
<td>0.342±0.008#</td>
<td>0.371±0.008</td>
</tr>
<tr>
<td>Cross-sectional area of the intima media of aorta (mm²)</td>
<td>0.924±0.042</td>
<td>1.053±0.033</td>
<td>0.968±0.056</td>
<td>0.964±0.067ψ</td>
<td>0.957±0.030</td>
</tr>
<tr>
<td>Cross-sectional area of the intima media of aorta (mm²)/100 g BW</td>
<td>0.257±0.009</td>
<td>0.322±0.011*</td>
<td>0.285±0.016</td>
<td>0.270±0.020ψ</td>
<td>0.283±0.010</td>
</tr>
</tbody>
</table>

BP=blood pressure, BW=body weight, HW=heart weight
* P<0.05 versus untreated
** P<0.01 versus untreated, losartan, and Ang II+losartan II
† P<0.05 versus untreated, Ang II and Ang II+PD 123319
# P<0.05 versus Ang II and Ang II+PD 123319

Significant by F-test only. ψ P<0.05 versus untreated, ψ P<0.05 versus Ang II
TABLE 2. Morphometric Parameters of Small Arteries

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Ang II</th>
<th>Losartan</th>
<th>Ang II + Losartan</th>
<th>Ang II + PD123319</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coronary arteries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>248±5</td>
<td>244±11</td>
<td>251±10</td>
<td>254±13</td>
<td>251±5</td>
</tr>
<tr>
<td>Media width (μm)</td>
<td>89±0.3</td>
<td>12.5±0.3**</td>
<td>93±0.3</td>
<td>92±0.3</td>
<td>11.6±0.4**</td>
</tr>
<tr>
<td>Media/lumen (%)</td>
<td>36±0.2</td>
<td>51±0.4*</td>
<td>37±0.2</td>
<td>37±0.3</td>
<td>46±0.2*</td>
</tr>
<tr>
<td><strong>Renal arteries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>249±13</td>
<td>243±12</td>
<td>243±11</td>
<td>237±12</td>
<td>265±15</td>
</tr>
<tr>
<td>Media width (μm)</td>
<td>89±0.2</td>
<td>12±0.3**</td>
<td>87±0.3</td>
<td>98±0.3</td>
<td>12.9±0.4**</td>
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<tr>
<td>Media/lumen (%)</td>
<td>36±0.1</td>
<td>50±0.3*</td>
<td>36±0.2</td>
<td>41±0.3</td>
<td>49±0.3*</td>
</tr>
<tr>
<td><strong>Mesenteric arteries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>234±10</td>
<td>239±7</td>
<td>240±10</td>
<td>239±7</td>
<td>224±6</td>
</tr>
<tr>
<td>Media width (μm)</td>
<td>88±0.3</td>
<td>12±0.3**</td>
<td>82±0.2</td>
<td>96±0.3</td>
<td>12.5±0.4**</td>
</tr>
<tr>
<td>Media/lumen (%)</td>
<td>36±0.1</td>
<td>52±0.3*</td>
<td>35±0.1</td>
<td>40±0.1</td>
<td>55±0.3*</td>
</tr>
<tr>
<td><strong>Femoral arteries</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>255±8</td>
<td>267±7</td>
<td>259±11</td>
<td>249±10</td>
<td>266±14</td>
</tr>
<tr>
<td>Media width (μm)</td>
<td>93±0.3</td>
<td>117±0.3**</td>
<td>92±0.3</td>
<td>94±0.2</td>
<td>12.4±0.3**</td>
</tr>
<tr>
<td>Media/lumen (%)</td>
<td>37±0.1</td>
<td>46±0.3</td>
<td>36±0.2</td>
<td>38±0.2</td>
<td>47±0.3*</td>
</tr>
</tbody>
</table>

*P<0.05  **P<0.01 versus untreated, Losartan, or Ang II+Losartan II

Antagonist, reversal to an immature phenotype, as shown by expression of cellular fibronectin and nonmuscle myosin, which was associated with Ang II–dependent growth of vascular smooth muscle cells, was abolished when losartan was administered with Ang II. Administration of losartan alone to normal rats reproduced the Ang II–induced vascular hypertrophy. The authors concluded that Ang II–induced growth of vascular smooth muscle cells is mediated by AT2 receptors, whereas the phenotypic changes of smooth muscle cells are AT1-dependent. The present results, particularly the cardiac and the small artery data, are in contrast to the results of Lévy et al and Sabri et al. They agree with previous evidence in favor of a role of AT1 receptors in growth of vascular smooth muscle cells, leaving open the possibility of an antiproliferative and pro-apoptotic action of AT2 receptors.

Cardiac weight and the volume per unit length (cross-sectional area) of the intima media of the aorta were significantly increased in angiotensin II–infused rats only when calculated relative to body weight, which was lower in this group. Hypertensive rats very often exhibit lower body weight than normotensive controls, and cardiac and vascular growth may not be apparent unless normalized by body weight. However, Brunk et al. have reported that angiotensin II induces weight loss through a pressor-independent mechanism that involves decreased food intake and body fat. This confounding factor has to be borne in mind, and the data regarding cardiac and aortic growth should be evaluated in the perspective that decreased adiposity resulting from reduced food intake may have affected these tissues differently from other tissues. In a previous study, we showed that in rats, aorta segment wet weight correlates with body weight and cross-sectional area of aorta correlates with the weight of aorta segments but is a more precise measurement. The cross-sectional area of the intima media of the aorta was lower in losartan–treated rats infused with Ang II than in Ang II–infused rats, achieving significance (P<0.05) only after a t-test but not by ANOVA. Interestingly, the cross-sectional area of the aorta (corrected or not for body weight) of rats treated only with losartan or infused simultaneously with Ang II and with the AT2 antagonist PD123319 achieved similar values (an increase of 4% to 5% above control), which were not statistically different from those in either untreated or Ang II–infused rats but were intermediate between these two groups. This is somewhat similar to the report of aortic growth in Ang II–infused rats by Lévy et al and Sabri et al., who found an increase of 5% in media thickness of aorta in Ang II–infused rats receiving PD123319 and an increase of 10% in rats receiving only losartan. The absence of statistical significance between some of these differences in mean values may be the result of a type II error, since the very limited amounts of the AT2 antagonist available made it possible for only very small numbers of rats to be evaluated under treatment for 3 weeks. With the large amounts of PD123319 required for a 3-week-long treatment at the dose of this agent that had been employed in the previous studies, not enough was available to study a group receiving only PD123319 or a combined group receiving losartan and PD123319. The potential explanation that AT2 receptors may play a modest growth-promoting role in aorta, as suggested by Lévy et al. and Sabri et al., cannot be conclusively ruled out in the present study, especially considering other independent evidence showing the
presence of AT$_2$ receptors in aortic smooth muscle and a hypertrophic effect mediated via AT$_2$ receptors in a smooth muscle layer derived from aorta. However, the present results suggest that in the heart and in small resistance arteries of four vascular beds, Ang II induces growth mainly via AT$_1$ receptors and that AT$_2$ receptors do not appear to be mediating growth-promoting effects at this level in this experimental paradigm.

The mechanism whereby angiotensin II exerts its growth-stimulating effect via AT$_1$ receptors has been in part elucidated recently when some studies have provided evidence that in other tissues, in blood vessels Ang II stimulates endothelin-1 production. Rajagopalan et al$^{20}$ demonstrated that after Ang II infusion for 5 days in rats, endothelin expression in the deeper smooth muscle layers of the aorta is enhanced, and blood pressure increase and aortic growth are as well antagonized by the AT$_1$ antagonist losartan as by an ET$_A$-selective endothelin receptor antagonist$^{21}$ showed that another ET$_A$ antagonist slightly reduced blood pressure and abrogated growth of mesenteric small arteries. Thus, smooth muscle expression of endothelin-1 appears to mediate in rats via ET$_A$ receptors the effects of exogenous angiotensin II infusion, the latter stimulating endothelin-1 through AT$_1$ receptor activation. It is possible that this AT$_1$ receptor-mediated involvement of endothelin-1 in Ang II-induced vascular growth in Ang II-infused rats occurs mainly in response to exogenous Ang II, since in 2-kidney 1 clip Goldblatt hypertensive rats, a high renin (and therefore Ang II) hypertensive model, vascular growth is relatively minor, with eutrophic rather than hypertrophic small artery remodeling predominating, and endothelin-1 does not appear to play an important role. In this study, as in previous studies examining small artery structure in the Ang II-infused rat, hypertrophic remodeling occurred with no reduction in lumen diameter of the resistance arteries examined. This remodeling is similar to the one that has been attributed to endothelin, whereas in hypertensive models in which endothelin is not involved but in which endogenous Ang II may play a role, the remodeling of small arteries is inward eutrophic, with reduction of the lumen diameter, as found in 2-kidney 1 clip Goldblatt hypertensive rats and in spontaneously hypertensive rats.$^{22}$

The absence of effects of the AT$_2$ antagonist in the present study could be due to ineffective blockade of AT$_2$ receptors by the dose of PD123319 used. However, the dose used in the present study is a very large one, and it is that infused by Lévy et al$^{23}$ and Sabri et al$^{24}$ to postulate effects mediated by AT$_2$ receptors in the same experimental paradigm. It is not easy to demonstrate blockade of AT$_2$ receptors unless binding inhibition with the pharmacological characteristics of AT$_2$ receptor binding is shown, since known physiological and biochemical effects of AT$_2$ receptors are presently mostly in vitro effects with few documented in vivo results. We are therefore unable in this study to certify that the dose that was used indeed achieved a blockade of putative AT$_2$ receptors to the same degree as in the previous studies, even if the dose used was the same, and this must be mentioned as a potential limitation of the study. The very large dose of PD123319 that had to be used in vivo for 3 weeks and the very limited availability of the compound precluded both the evaluation of larger numbers of rats in the PD123319-treated group and the introduction of two experimental groups that would be very useful for this study, that is, a group treated only with PD123319 and one receiving Ang II, losartan, and PD123319. These are recognized limitations of the study, since an appropriate baseline for comparison of the Ang II-infused PD123319-treated group is one receiving only PD123319.

In conclusion, these results support the concept that in Wistar normotensive rats infused for 3 weeks with angiotensin II, growth in the heart, aorta, and coronary, renal, mesenteric, and femoral small arteries is mediated in large measure by the AT$_1$ receptor and show little evidence, particularly in heart and
small mesenteric arteries, of a role of AT2 receptors in mediating angiotensin II effects in this experimental paradigm.

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References

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