Coronary Hemodynamic and Ventricular Responses to Angiotensin Type 1 Receptor Inhibition in SHR Interaction With Angiotensin Type 2 Receptors

Jasmina Varagic, Dinko Susic, Edward D. Frohlich

Abstract—This study was designed to determine the effects of angiotensin II type 1 (AT₁) receptor inhibition on coronary hemodynamics and ventricular mass and hydroxyproline content and the additive effects of angiotensin II type 2 (AT₂) receptor inhibition in spontaneously hypertensive rats (SHR). The selective AT₁ receptor antagonist candesartan (10 mg/kg per day) was administered alone or in combination with the AT₂ receptor antagonist PD 123319 (50 mg/kg per day) for 12 weeks. Control SHR received placebo for the same period. Left and right ventricular coronary blood flow, blood flow reserve, and minimal coronary vascular resistance were determined by using radiomicrospheres in male 35-week-old rats. Mean arterial pressure; total peripheral resistance; left and right ventricular, renal, and aortic weights; and hydroxyproline concentration were also determined. Candesartan reduced mean arterial pressure and left ventricular, renal, and aortic masses, as well as hydroxyproline concentration and minimal coronary vascular resistance of both ventricles. PD 123319 partially prevented the hypotensive effect of AT₁ receptor inhibition and reversed the effect on myocardial hydroxyproline concentration. These data suggest that AT₂ receptors contribute to the hypotensive and antifibrotic effects but not the coronary hemodynamic improvement or reduced left ventricular mass of AT₁ receptor inhibition in these adult SHR. (Hypertension. 2001;37:1399-1403.)

Key Words: angiotensin II receptors n blood pressure n hemodynamics n rats, inbred SHR n fibrosis

Angiotensin II type 1 (AT₁) receptor agonism is responsible for most actions of angiotensin II on arterial pressure, including arteriolar constriction, increased myocardial contractility, increased renal sodium and water retention, and cardiovascular myocyte and fibrocyte mitogenesis. In recent years, a large body of evidence demonstrating that angiotensin II acts not only through AT₁ but also through angiotensin II type 2 (AT₂) receptors has evolved.¹⁻⁴ Although AT₂ receptor mRNA expression rapidly diminishes, or even disappears in various tissues and organs in the early postnatal period,⁵⁻⁷ AT₂ receptor protein remains detectable in adult heart, vasculature, and kidney.⁵⁻⁹ Moreover, AT₂ receptor expression can be modulated by pathological states associated with tissue remodeling or certain experimental maneuvers.¹⁰⁻¹² Currently, it is believed that AT₂ receptors act reciprocally to modulate the opposing effects of AT₁ receptors on cardiac and vascular myocytic and fibrocytic mitogenesis as well as in cellular differentiation and arterial pressure regulation.¹³⁻¹⁵

Acute and chronic inhibition of AT₁ receptors reduces arterial pressure and improves systemic and coronary hemodynamics in spontaneously hypertensive rats (SHR).¹⁶⁻¹⁹ Numerous studies have shown that AT₁ receptor antagonists are also effective in reducing left ventricular (LV) mass and fibrosis.¹⁸⁻²⁰ These findings suggest that unopposed AT₂ receptor action might participate during selective AT₁ receptor inhibition, thereby contributing to some of the beneficial effects in the SHR and other experimental models of hypertension.²¹,²² Thus, the present study was designed to determine the contribution of AT₂ receptors associated with prolonged AT₁ antagonism in the SHR.

Methods

Procedures
Male 16-week-old SHR obtained from Charles River Breeding Laboratories Inc (Wilmington, Mass) were maintained in a temperature- and light-controlled room. All had free access to standard rat chow and tap water and were handled in accordance with National Institutes of Health guidelines, and the protocol followed was approved in advance by our institutional Animal Care and Use Committee.

At 22 weeks of age, the rats were divided randomly into 3 groups. They received the selective AT₁ receptor antagonist candesartan (10 mg/kg per day) either alone (SHR-C group, n=14) or in combination with the selective AT₂ receptor antagonist PD 123319 (50 mg/kg per day; SHR-C+PD group, n=8) for 12 weeks. Control SHR received placebo (SHR-P group, n=12) for the same duration. Candesartan was suspended in 5% gum arabic solution and was given by daily gastric gavage. An osmotic minipump (model 2 ML4, Alzet) was implanted subcutaneously with the animals under pentobarbital

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anesthesia (40 mg/kg IP) for delivery of PD 123319 dissolved in saline solution. This osmotic minipump was replaced with a new one every 4 weeks. After 12 weeks of treatment, the rats were anesthetized with pentobarbital (40 mg/kg), and their systemic and regional hemodynamics were determined by using the reference standard microsphere method as described previously.23–25 In brief, a jugular vein, femoral artery, and the LV (via right carotid artery) were cannulated with polyethylene catheters (PE-50) and exteriorized at the nape of the neck through a subcutaneous tunnel. Baseline measurements of systemic and regional hemodynamics were obtained from the unrestrained rats after full recovery from anesthesia by injecting radioactively labeled microspheres (113mCo). To this end, the femoral arterial catheter was connected to a pressure transducer (P23Db, Statham Instruments), and mean arterial pressure (MAP) was recorded on a multichannel physiograph (Sensor Medics R612) while the heart rate was simultaneously derived through a tachometer coupler. The same arterial catheter was used to collect blood for hematocrit determination (by capillary microcentrifugation). Cardiac output was measured by the reference sample microsphere method,23–25 and cardiac index (CI) was calculated from cardiac output and body weight and expressed as mL/min per kilogram. Total peripheral resistance index (U/kg) was calculated by dividing MAP by CI.

After these basal measurements were obtained, maximal coronary vasodilatation was achieved by dipyridamole infusion (4 mg/kg per minute IV for 10 minutes).16,25 The hemodynamic studies were repeated by using a second microsphere radiouclide (113mSm). At the end of each study, the rat was killed with pentobarbital overdose, and immediately thereafter, the heart, aorta, lungs, liver, brain, kidneys, and samples of skin and skeletal muscle were removed. After cardiac removal, the atria were dissected free from the ventricles and discarded; and the free wall of the right ventricle (RV) was separated carefully from the LV (the septum remaining with LV). Ventricular weights were recorded and were normalized for body weight and expressed as ventricular mass indices (mg/g). A 3-cm-long segment of the descending aorta (starting from a point just distal to the origin of the subclavian artery) was also removed, weighed, normalized for its length and body weight, and expressed as aortic mass index. Tissue samples, as well as blood reference samples, were placed in plastic scintillation vials and counted for 15 minutes in a deep-well γ-scintillation spectrometer (Packard Instruments) with a multichannel analyzer. Organ blood flows were calculated by multiplying the fractional distribution of radioactivity to each organ by cardiac output and were normalized for wet weight (mL/min per gram). Coronary flow reserve for each ventricle was calculated as the difference between flows during the baseline and dipyridamole infusion flows. Organ vascular resistances were calculated by dividing MAP by the respective organ flow; they were normalized for organ weight and expressed as U/g. Minimal coronary vascular resistance (CVRmin) was defined as that vascular resistance achieved by CI.

Myocardial Collagen Content
As an estimate of ventricular collagen content, hydroxyproline concentration was determined for both the LV and RV samples, as previously described,23 and expressed as mg/g dry wt.

Statistical Analysis
A 1-way ANOVA and Student-Newman-Keuls post hoc tests were used to test for significant differences between groups.27 All values are expressed as the mean±1 SEM. A 5% confidence level was considered to be of statistical significance.

Results
Body weight was significantly lower in SHR-C (358±3 g) than in SHR-P (400±4 g); PD 123319 prevented this effect (389±8 g in SHR-C+PD, P<0.05). LV and aortic mass indices were significantly (P<0.05) reduced by candesartan, and similar responses were achieved with the simultaneous inhibition of AT1 and AT2 receptors (Figure 1). RV mass was not different among the 3 groups. Renal mass index was reduced in those rats receiving candesartan; this was also prevented by PD 123319 (Figure 1). AT1 receptor inhibition reduced hematocrit compared with hematocrit in SHR-P (41±1.5% versus 50±0.6%, P<0.05), and this was reduced further in those SHR-C+PD (36±1.1%, P<0.05).

Figure 1. Effects of candesartan (SHR-C [C] group) and candesartan with PD 123319 (SHR-C+PD [C +PD] group) on ventricular, aortic, and renal mass indices. P indicates control SHR-P group. Values are mean±1 SEM. *P<0.05 compared with SHR-P; †P<0.05 compared with SHR-C.

Candesartan was extremely effective in reducing MAP associated with a significant reduction in total peripheral resistance. This was partially prevented by PD 123319 (Figure 2). Heart rate remained unaffected by AT1 or AT2, and AT2 receptor inhibition. CI remained unchanged in rats treated with candesartan, but with concomitant blockade of AT1 and AT2 receptors, CI was increased, resulting in no differences in total peripheral resistance between these 2 groups (Figure 2).

There were no differences in baseline right and left coronary hemodynamics among the 3 groups, although rats...
receiving candesartan and PD 123319 had slightly greater baseline coronary blood flow (Table 1). Baseline coronary vascular resistance (CVR) of both ventricles was significantly reduced in SHR-C and SHR-C+PD. Of particular interest, both LV and RV coronary flow reserves were significantly increased by candesartan. Furthermore, AT1 receptor inhibition alone or with simultaneous antagonism of AT2 receptors (Table 1). These regional hemodynamic parameters remained unchanged by the simultaneous inhibition of the AT1 and AT2 receptors (Table 2), except that concomitant inhibition of the AT1 and AT2 receptors increased blood flow and decreased vascular resistance in skin (Table 2).

Also of major significance was the reduced hydroxyproline concentration in both the liver and skin with candesartan treatment. Notably, this was prevented by concomitant inhibition of AT2 receptors (Figure 3).

**Discussion**

The results of the present study demonstrate that candesartan is extremely effective in correcting the adverse cardiovascular effects of hypertension in SHR, as manifested by reduction of arterial pressure to a normotensive level and improvement of systemic as well as coronary hemodynamics. These findings are consistent with previous reports from other laboratories, but most notable in this respect was the reduction in arterial pressure to the lowest levels, which was not observed with any other antihypertensive agent. Of particular interest in the present study was that simultaneous inhibition of AT1 with AT2 receptors partially prevented this optimal reduction of pressure achieved by AT1 blockade alone, suggesting that stimulation of unopposed AT2 receptors by reportedly increased plasma angiotensin II levels was responsible, at least in part, for the hypotensive effect of AT1 receptor antagonism. Earlier discovery of the putative vasodilating effects of AT2 receptor activation via the bradykinin-NO-cGMP cascade gives further support to our observation. Thus, the present study elucidates the important role of AT2 receptors in the overall hypotensive effect of AT1 inhibition in SHR, a finding already shown in angiotensin II or renal-encapsulation hypertension. Additionally, a slight reduction in hematocrit by candesartan might also participate in the fall in arterial pressure in this experimental group, although a significant degree of anemia

**TABLE 1. LV and RV Coronary Hemodynamic Indices in SHR-P, SHR-C, and SHR-C+PD**

<table>
<thead>
<tr>
<th></th>
<th>SHR-P (n=11)</th>
<th>SHR-C (n=13)</th>
<th>SHR-C+PD (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV CBF, mL/min/g</td>
<td>5.452±0.364</td>
<td>5.578±0.891</td>
<td>7.473±0.556</td>
</tr>
<tr>
<td>LV CVR, U/g</td>
<td>33.760±2.471</td>
<td>23.205±3.311*</td>
<td>15.830±1.803*</td>
</tr>
<tr>
<td>LV CVRmin, U/g</td>
<td>15.722±1.642</td>
<td>6.570±0.779*</td>
<td>7.113±1.139*</td>
</tr>
<tr>
<td>LV CFR, mL/min/g</td>
<td>4.847±0.864</td>
<td>9.505±1.286*</td>
<td>7.970±1.750</td>
</tr>
<tr>
<td>RV CBF, mL/min/g</td>
<td>4.353±0.593</td>
<td>4.085±0.492</td>
<td>5.634±0.648</td>
</tr>
<tr>
<td>RV CVR, U/g</td>
<td>47.732±5.983</td>
<td>30.318±4.674*</td>
<td>22.191±2.696*</td>
</tr>
<tr>
<td>RV CVRmin, U/g</td>
<td>18.522±2.697</td>
<td>7.160±0.846*</td>
<td>7.301±1.041*</td>
</tr>
<tr>
<td>RV CFR, mL/min/g</td>
<td>5.504±0.929</td>
<td>9.102±1.061*</td>
<td>7.640±1.075</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. CBF indicates coronary blood flow; CFR, coronary blood flow reserve. *P<0.05 vs SHR-P; †P<0.05 vs SHR-C.

**TABLE 2. Organ Blood Flow and Vascular Resistance After 12-wk Treatment**

<table>
<thead>
<tr>
<th></th>
<th>SHR-P (n=11)</th>
<th>SHR-C (n=13)</th>
<th>SHR-C+PD (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ blood flow, mL/min/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>0.104±0.012</td>
<td>0.085±0.008*</td>
<td>0.173±0.021†</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.094±0.007</td>
<td>0.093±0.012</td>
<td>0.113±0.008</td>
</tr>
<tr>
<td>Brain</td>
<td>1.327±0.142</td>
<td>1.461±0.169</td>
<td>1.513±0.157</td>
</tr>
<tr>
<td>Liver</td>
<td>0.233±0.038</td>
<td>0.106±0.028*</td>
<td>0.086±0.007*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>7.514±0.418</td>
<td>12.307±0.748*</td>
<td>13.433±0.841*</td>
</tr>
<tr>
<td>Organ vascular resistance, U/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>1894±212</td>
<td>1347±201*</td>
<td>743±83†</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1998±175</td>
<td>1233±131*</td>
<td>1076±85*</td>
</tr>
<tr>
<td>Brain</td>
<td>150.71±20.92</td>
<td>78.43±9.62*</td>
<td>80.70±8.40*</td>
</tr>
<tr>
<td>Liver</td>
<td>1001±168</td>
<td>1732±376</td>
<td>1481±207</td>
</tr>
<tr>
<td>Kidneys</td>
<td>24.156±1.648</td>
<td>8.428±0.708*</td>
<td>9.005±0.659*</td>
</tr>
</tbody>
</table>

Values are mean±1 SEM. *P<0.05 vs SHR-P; †P<0.05 vs SHR-C.
was not produced. Naeshiro et al.\(^2^7\) have suggested that inhibition of AT\(_1\) receptors increased renal blood flow, which, in turn, suppressed erythropoietin production and thereby induced anemia. Because candesartan could have been responsible for the hematocrit decrease by blocking erythropoietin production, we explored this possibility by studying the 2 groups of rats exposed to hypoxemia and given 1 of 2 single doses of candesartan (5 and 10 mg/kg). Candesartan did not directly affect hypoxia-induced erythropoietin production (91±16 mU/mL in controls; 0.151±0.1 and 141±34 mU/mL in doses of 5 and 10 mg/kg, respectively) with these 2 doses (J. Fisher, unpublished data, 2000). Furthermore, our additional data that PD 123319 decreased hematocrit further (compared with candesartan alone) suggested that AT\(_2\) receptor stimulation during AT\(_1\) receptor inhibition partially prevented the fall in hematocrit. Therefore, it appears that the mechanism of anemia induced by agents interfering with the renin-angiotensin system\(^3^8,3^9\) requires further investigation.

Another new and important finding in the present study is that the AT\(_2\) receptors did not contribute to the improved coronary hemodynamics associated with AT\(_1\) receptor blockade in SHR. Candesartan improved both LV and RV hemodynamics, and it reduced LV mass. These findings suggest that the hemodynamic action of AT\(_1\) receptor inhibition appears to be independent of its effect on ventricular mass, a finding that we also observed with losartan,\(^1^6\) certain ACE inhibitors,\(^3^1,4^0\) calcium antagonists,\(^2^5\) clonidine,\(^3^3\) and certain \(\beta\)-adrenergic receptor inhibitors.\(^4^1\)

The present study demonstrates that AT\(_1\) receptor inhibition decreased hydroxyproline concentration in both ventricles, and this action was prevented when PD 123319 was administered concomitantly. Although the present study did not attempt to determine the mechanism of the role of angiotensin receptors on ventricular hydroxyproline concentration, it appears that because there were parallel changes in hydroxyproline concentration in both ventricles, the development or reversal of myocardial fibrosis is not necessarily dependent on pressure overload. Previous reports from our and other laboratories have already shown dissociation of changes in hemodynamics, ventricular mass, and fibrosis with different classes of antihypertensive drugs.\(^2^5,4^2\) Furthermore, earlier studies have clearly demonstrated that angiotensin-II stimulates collagen synthesis in cultured adult rat cardiac fibroblasts via AT\(_1\) receptors,\(^4^3,4^4\) whereas the role of AT\(_2\) receptors has not been as well established.\(^1^3,4^3,4^5\) The present results agree with previous reports that AT\(_2\) receptor stimulation inhibits the growth of cardiac fibroblasts.\(^4^5,4^7\) Our findings of the potential role of AT\(_2\) receptor activation in reducing ventricular fibrosis during AT\(_1\) receptor antagonism suggest an important clinical and therapeutic relevance, inasmuch as increased ventricular collagen content would favor diastolic dysfunction and congestive heart failure in patients with hypertension.\(^4^8\) Moreover, because AT\(_1\) receptors may be up-regulated in cardiac fibroblasts in the failing human heart,\(^4^9\) selective stimulation of AT\(_2\) could provide the valuable cardioprotective feature of AT\(_1\) blockade in patients with or predisposed to cardiac failure.\(^5^0\)

Finally, candesartan significantly reduced renal mass index, and the simultaneous blockade of AT\(_1\) receptors prevented this effect. This finding suggests that stimulation of unopposed AT\(_2\) receptors during AT\(_1\) receptor inhibition participates in the reduction in renal mass and that angiotensin could have an important role in the regulation of renal growth.

In conclusion, the beneficial effect of prolonged candesartan treatment on arterial pressure and ventricular fibrosis but not on coronary hemodynamics and LV and aortic mass appears to be dependent not only on AT\(_1\) receptor antagonism but also on the selective activation of AT\(_2\) receptors.

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References
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