AT₂ Receptor Stimulation Enhances Antihypertensive Effect of AT₁ Receptor Antagonist in Hypertensive Rats

Melissa N. Barber, Donella B. Sampey, Robert E. Widdop

Abstract—In the present study, we investigated the role of the angiotensin type 2 (AT₂) receptor in the regulation of blood pressure in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). We tested the hypothesis that AT₂ receptor activation may contribute to the antihypertensive effects of angiotensin type 1 (AT₁) receptor antagonists. Mean arterial pressure (MAP) and heart rate were measured over a 4-day protocol in various groups of rats that received the following drug combinations: the AT₁ receptor antagonist candesartan (0.01 or 0.1 mg/kg IV) alone, the AT₂ receptor agonist CGP42112 (1 μg/kg per minute) alone, and candesartan plus CGP42112. In both SHR and WKY, 4-hour infusions of saline and CGP42112 alone did not alter MAP. In WKY, both doses of candesartan alone caused small decreases in MAP, which were similar when combined with CGP42112. In SHR, candesartan (0.1 mg/kg) caused an immediate, marked decrease in MAP, which was unaffected when combined with CGP42112. By contrast, in separate SHR, a 10-fold lower dose of candesartan (0.01 mg/kg) caused a slower-onset depressor response, which was enhanced when combined with CGP42112. The involvement of AT₂ receptors was confirmed in another group of SHR, since this facilitation of the antihypertensive effect of candesartan by CGP42112 was abolished by the coinfusion of the AT₂ receptor antagonist PD123319 (50 μg/kg per minute) with the candesartan/CGP42112 combination. Collectively, these data suggest that in SHR, AT₂ receptor activation can facilitate the initial depressor response caused by an AT₁ receptor antagonist. (Hypertension. 1999;34:1112-1116.)

Key Words: receptors, angiotensin ■ vasodilation ■ angiotensin II ■ hypertension, arterial ■ rats

Angiotensin II (Ang II), the main effector peptide of the renin-angiotensin system, plays an important role in blood pressure regulation and fluid homeostasis and is likely to play a role in the pathogenesis of hypertension.¹,² Ang II acts at 2 main receptor subtypes: angiotensin type 1 (AT₁) or angiotensin type 2 (AT₂). AT₁ receptors are widely distributed throughout the body, including vascular smooth muscle, kidney, heart, and brain. AT₂ receptors are responsible for mediating most of the known actions of Ang II, including vasoconstriction and aldosterone release.³ Not surprisingly, AT₁ receptor antagonists have already proven to be clinically effective antihypertensive agents.³ However, the role of the AT₂ receptor is less well defined. It is expressed in high levels in the developing fetus, which has led to the suggestion that the AT₂ receptor is involved in growth and development.⁴⁻⁷

Recently, a number of studies have implicated the AT₂ receptor as having an opposing role to the AT₁ receptor in certain experimental settings, including endothelial cell proliferation and neointimal formation. In both situations, the AT₁ receptor causes stimulation, while the AT₂ receptor mediates inhibition of the response.⁵,⁷,⁸ Similarly, we have recently reported that AT₂ receptor blockade increased AT₁ receptor–mediated contraction in the rat isolated ureter artery.⁹ Studies in transgenic mice have also suggested an inhibitory role of the AT₂ receptor in blood pressure control since basal blood pressure and/or pressor sensitivity evoked by Ang II was increased in mice when the AT₂ receptor gene had been disrupted.¹⁰,¹¹

Importantly, AT₁ receptor antagonists are associated with a rise in plasma Ang II concentration due to the inhibition of the AT₁ receptor–mediated negative feedback on renin release.³,⁶,¹² Therefore, it has been suggested that, at therapeutic doses of AT₁ receptor antagonists, endogenous Ang II may stimulate unopposed AT₂ receptors and thereby contribute to the decrease in blood pressure.⁶ However, in vivo evidence for this hypothesis is mainly indirect since it is based on enhanced Ang II–mediated vasoconstriction in the presence of AT₂ receptor blockade.¹⁰,¹¹,¹³,¹⁴

Another approach has been to infuse Ang II in the presence of AT₁ receptor blockade to stimulate AT₂ receptors.¹⁴,¹⁵ In 1 study it was claimed that, in normotensive rats, there was a greater antihypertensive effect of losartan when combined with Ang II than compared with losartan alone, although the dose of losartan alone was 10-fold less than the drug combination, which makes any interpretation difficult.¹⁴ In another study, AT₂ receptor stimulation increased aortic cyclic GMP content; however, any potential blood pressure changes may have been masked by

Received April 15, 1999; first decision May 20, 1999; revision accepted June 29, 1999.
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direct vasoconstriction caused by infusion of a large dose of Ang II alone.\textsuperscript{15}

Therefore, in the present study we determined whether selective AT\textsubscript{2} receptor stimulation in vivo can alter blood pressure. In so doing, we tested the hypothesis that AT\textsubscript{2} receptor–mediated vasodilatation may contribute to the antihypertensive effects of AT\textsubscript{1} receptor antagonists. For this purpose, we used the highly specific AT\textsubscript{2} receptor ligand CGP42112,\textsuperscript{16} which has been shown to act as an agonist in both studies using cells specifically expressing AT\textsubscript{2} receptors\textsuperscript{17,18} and functional studies.\textsuperscript{7,9,19} Importantly, CGP42112 does not exert any cardiovascular effects at appropriate doses.\textsuperscript{20,21} Therefore, we determined the antihypertensive effect of the AT\textsubscript{1} receptor antagonist candesartan,\textsuperscript{22–24} in the absence and presence of CGP42112, in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY).

**Methods**

Male SHR and WKY, weighing 350 to 400 g and aged \(\sim\)16 to 18 weeks, were obtained from the Austin Hospital Research Laboratories and were maintained on a 12-hour day/night cycle with free access to food and water.

**General Procedures**

Rats were anesthetized with methohexitone sodium (60 mg/kg IP, supplemented as required). A catheter was inserted into the right carotid artery for measurement of blood pressure, and 2 catheters were implanted into the right jugular vein for intravenous drug administration.

Experiments were performed 24 to 48 hours after surgery in conscious, unrestrained rats. Arterial blood pressure, measured directly via the arterial catheter attached to a pressure transducer (Gould Inc), was recorded with the use of a MacLab–8 data acquisition system (ADInstruments) interfaced with a Macintosh computer. Heart rate (HR) and mean arterial pressure (MAP) were derived from the phasic blood pressure signal.

**Experimental Protocols**

The AT\textsubscript{1} receptor antagonist candesartan was given to separate groups of rats as an intravenous bolus at 2 doses (0.01 and 0.1 mg/kg), on the basis of previous studies.\textsuperscript{23,24} The AT\textsubscript{2} receptor agonist CGP42112 was given as an infusion at 1 \(\mu\)g/kg per minute for 4 hours, which was previously shown to be highly selective for AT\textsubscript{2} receptors.\textsuperscript{20,21} The AT\textsubscript{2} receptor antagonist PD123319 was given by bolus injection at 50 \(\mu\)g/kg per minute for 2 hours, on the basis of previous studies.\textsuperscript{19,25}

Basal MAP and HR were recorded over a 4-day protocol in 5 separate groups of rats, as outlined below. In all groups, on day 1, rats received a 4-hour infusion (\(\sim\)1 mL/kg per hour IV) of saline (0.9% NaCl). Group 1 involved candesartan (0.1 mg/kg) with or without CGP42112 infusion in WKY. On the subsequent 3 days, WKY were randomized to receive (1) candesartan (0.1 mg/kg IV) plus a saline infusion for 4 hours; (2) candesartan (0.1 mg/kg IV) plus an infusion of CGP42112 (1 \(\mu\)g/kg per minute) for 4 hours; and (3) a 4-hour infusion of CGP42112 alone (1 \(\mu\)g/kg/min). In group 2, a protocol identical to that in group 1 was performed, but SHR were used. In groups 3 and 4, in separate groups of WKY and SHR, protocols identical to those in groups 1 and 2 were repeated, but a 10-fold lower dose of candesartan (0.01 mg/kg IV) was used. Group 5 involved candesartan (0.01 mg/kg IV) with or without CGP42112 and PD123319 infusions in SHR. In a separate group of SHR, after the control saline day, rats were randomized to receive (1) candesartan (0.01 mg/kg IV) plus a saline infusion for 4 hours; (2) candesartan (0.01 mg/kg IV) plus an infusion of CGP42112 (1 \(\mu\)g/kg per minute) for 4 hours; and (3) candesartan (0.01 mg/kg IV) plus an infusion of CGP42112 (1 \(\mu\)g/kg per minute) for 4 hours and a 2-hour infusion of PD123319 (50 \(\mu\)g/kg per minute).

**Drugs**

Candesartan was a gift from Takeda Chemical Industries (Japan), and PD123319 was a gift from Dr J. Keiser, Parke-Davis, Ann Arbor, Mich. Ang II and CGP42112 were purchased from Auspep and Bachem, respectively.

**Statistical Analysis**

All data are presented as mean\(\pm\)SEM. Changes in MAP and HR from baseline on any given treatment day were analyzed with 1-way ANOVA with repeated measures. Differences between treatments and treatment/time interactions were analyzed with 2-way ANOVA with repeated measures. Statistical significance was accepted as \(P<0.05\).

**Results**

The baseline MAP and HR, over the 4 experimental days, in all groups of SHR and WKY are listed in Tables 1 and 2. Given that these values were similar over the experimental

**TABLE 1. Resting MAP and HR Recorded on Separate Days Before Treatment Indicated, in WKY**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>130±4</td>
<td>311±22</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg)</td>
<td>114±2</td>
<td>307±16</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>120±5</td>
<td>315±10</td>
</tr>
<tr>
<td>Group 3 (n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>133±5</td>
<td>302±10</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg)</td>
<td>111±4</td>
<td>320±8</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>121±5</td>
<td>309±7</td>
</tr>
</tbody>
</table>

Values are mean\(\pm\)SEM.

**TABLE 2. Resting MAP and HR Recorded on Separate Days, Before Treatment Indicated, in SHR**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2 (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>174±8</td>
<td>318±13</td>
</tr>
<tr>
<td>Candesartan (0.1 mg/kg)</td>
<td>155±7</td>
<td>310±10</td>
</tr>
<tr>
<td>Candesartan (0.1 mg/kg) + CGP42112</td>
<td>172±7</td>
<td>319±10</td>
</tr>
<tr>
<td>Group 4 (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>175±4</td>
<td>321±6</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg)</td>
<td>159±6</td>
<td>321±10</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>166±5</td>
<td>311±14</td>
</tr>
<tr>
<td>Group 5 (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>164±5</td>
<td>327±10</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>179±8</td>
<td>313±13</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + PD123319</td>
<td>168±4</td>
<td>308±7</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + PD123319 + CGP42112</td>
<td>166±6</td>
<td>310±14</td>
</tr>
</tbody>
</table>

Values are mean\(\pm\)SEM.
period, these data indicate that individual daily treatments did not alter MAP and HR on subsequent days. Generally, the effects of various treatments had minimal effects on HR, except for tachycardia (20 to 40 bpm) in response to candesartan (data not shown).

In both rat strains, infusion of saline or CGP42112 (1 mg/kg per minute) alone had no effect on MAP (Figures 1 to 4). At the higher dose, candesartan (0.1 mg/kg) lowered MAP in WKY and SHR (both \( P < 0.01 \), ANOVA), although the effect was greater in the latter group (Figures 1 and 2). Similarly, candesartan, combined with an infusion of CGP42112 (1 mg/kg per minute) for 4 hours, decreased MAP in both strains (both \( P < 0.01 \), ANOVA), although these depressor responses were not significantly different from those for candesartan alone in the respective groups (\( P > 0.05 \), ANOVA; Figures 1 and 2).

An identical 4-day protocol was performed in separate groups of SHR and WKY but with a 10-fold lower dose of candesartan (0.01 mg/kg), since it was possible that the immediate and marked antihypertensive effect of candesartan at the higher dose may have masked more subtle effects when combined with CGP42112. In WKY, candesartan (0.01 mg/kg), with or without CGP42112, caused small reductions in MAP (Figure 3), as observed previously. By contrast, candesartan (0.01 mg/kg) alone caused a slow but progressive fall in MAP in SHR (\( P < 0.01 \), ANOVA; Figure 4). Furthermore, the combination of candesartan (0.01 mg/kg) with a CGP-42112 infusion caused a faster-onset decrease in MAP that was sustained for the duration of CGP-42112 infusion (Figure 4). This difference in the time course of antihypertensive effect of candesartan resulted in a significant treatment/time interaction (\( P < 0.05 \), ANOVA).

Given this synergistic effect, another group of SHR was given CGP42112 and candesartan, and a 2-hour infusion of PD123319 (50 mg/kg per minute) was also included as part of the experimental protocol (Figure 5). As in the previous group of SHR, CGP42112 facilitated the antihypertensive effect of candesartan (0.01 mg/kg) compared with candesartan alone (\( P < 0.05 \), ANOVA). However, PD123319 markedly attenuated the antihypertensive effect of the candesartan/CGP42112 combination (\( P < 0.05 \), ANOVA; Figure 5), such that the combination of all 3 drugs decreased MAP in a manner similar to that of candesartan alone (\( P > 0.05 \), ANOVA). Moreover, MAP decreased further after the PD123319 infusion was stopped (Figure 5).
Moreover, results from this study suggest that the AT$_2$ receptor–mediated vasodilatation in conscious SHR. This study represents the first in vivo demonstration of direct pressure regulation, at least in SHR.

The main finding of the present study is that AT$_2$ receptor–mediated depressor responses were demonstrated, but only in the presence of AT$_1$ receptor blockade. To our knowledge, this study represents the first in vivo demonstration of direct AT$_2$ receptor–mediated vasodilatation in conscious SHR. Moreover, results from this study suggest that the AT$_2$ receptor opposes the action of the AT$_1$ receptor in blood pressure regulation, at least in SHR.

Previous studies using Ang II to stimulate AT$_2$ receptors have been complicated by the direct (AT$_1$ receptor–mediated) vasoconstrictor action of the peptide and/or inappropriate experimental designs, as noted. Therefore, in the present study CGP42112 was used as the AT$_2$ receptor agonist because it is devoid of cardiovascular effects at up to 100 times the dose used here. The AT$_2$ receptor antagonist candesartan is the potent, long-acting metabolite of candesartan cilexetil and causes prolonged inhibition of the vasoconstrictor effects of Ang II after a single intravenous dose.

At the higher dose used, candesartan (0.1 mg/kg) caused an immediate decrease in MAP in both SHR and WKY, although the depressor response was greater in the SHR than WKY, as demonstrated in previous studies. However, the combination of candesartan (0.1 mg/kg) and CGP42112 did not cause a further decrease in MAP. One possible explanation for this lack of additional depressor response may be that this dose of candesartan increased endogenous Ang II levels was not determined in this study. Conceivably, an interaction between CGP42112 and Ang II contributed to the differences seen with the 2 doses of candesartan. Importantly, the low-dose candesartan/CGP42112 combination data were confirmed in another group of SHR. Moreover, PD123319 infused (for 2 hours) with the candesartan/CGP42112 combination reversed the accelerated MAP drop, thus confirming the involvement of AT$_2$ receptor–mediated vasodilatation. Because of limited drug supplies, we did not infuse PD123319 alone. However, our own unpublished observations (M.N.B. et al, unpublished data, 1998), as well as other studies, indicate that, at this dose, PD123319 exerts no cardiovascular effects per se. Further support for an inhibitory role of the AT$_2$ receptor is indicated by the fact that at the end of the 4-hour candesartan/CGP42112 infusion, ie, 2 hours after the PD123319 infusion was stopped, MAP had again decreased substantially.

The present study has unequivocally demonstrated an AT$_2$ receptor–mediated depressor component in conscious SHR, which contrasts with studies using AT$_1$ receptor blockade combined with Ang II. Gohike et al found that the depressor effect caused by Ang II and losartan together was less than with losartan alone, presumably because the Ang II–induced pressor effect alone had offset the antihypertensive effect of losartan. However, the combination of losartan/Ang II and PD123319 tended to lower MAP more than losartan alone, at least initially. Discrepancies between the present and previous studies may relate to differences in drugs used, route/rate of drug administration, and the use of stroke-prone SHR in the latter case. Ang II caused a depressor effect in anesthetized normotensive rats in the presence of AT$_1$ receptor blockade. However, this report has not been confirmed, and such covert depressor activity is not observed after injections of Ang II during AT$_1$ receptor blockade in the conscious state. Although the present data are consistent with those of Munzenmaier and Greene, although that study was performed in salt-loaded rats.

Thus, our results support the emerging concept that an additional depressor effect due to AT$_2$ receptor stimulation during AT$_1$ receptor blockade may play a role in the beneficial effects of AT$_1$ receptor antagonists. In this context, it has recently been reported that the cardiovascular effects of the AT$_2$ receptor antagonist losartan were blocked by the coadministration of the AT$_1$ receptor antagonist PD123319 in rats with heart failure or in rats with sodium depletion. The fact that AT$_2$ receptor stimulation causes vasodilatation suggests that, in addition to the predominant AT$_1$ receptor subtype, there are also AT$_2$ receptors located in the vasculature. Indeed, early autoradiographic studies did in fact report that AT$_1$ receptors account for 40% of Ang II receptors in aortic tissue. AT$_2$ receptors have also been implicated in coronary endothelial cells and in skeletal muscle microvascularity. More recently, immunohistochemical studies have identified AT$_2$ receptors in endothelium and vascular smooth muscle of large and small microvessels, and mRNA expression for both AT$_1$ and AT$_2$ receptors was demonstrated in aortic tissue. Moreover, there was enhanced expression of both receptor subtypes in SHR compared with WKY.
Thus, there is increasing evidence indicating that AT₂ receptors are localized in close proximity to vascular AT₁ receptors. These anatomic findings are consistent with several recent studies suggesting that AT₂ receptor activation is linked to NO/cGMP production, presumably via the endothelium. The fact that AT₂ receptor stimulation in isolated vasculature leads to the production of cGMP further supports a pivotal role for the AT₂ receptor to oppose the excitatory effects of AT₁ receptor stimulation.

In conclusion, the potentiation of the initial antihypertensive effect that contributes to the therapeutic action of AT₁ receptor antagonists. Finally, this effect was observed in SHR but not in WKY, which may suggest that covert AT₂ receptor–mediated vasodilatation occurs as a consequence of hypertension and is consistent with enhanced expression of this subtype in SHR.

Acknowledgments
This work was supported in part by grants from the National Health and Medical Research Council of Australia and the National Heart Foundation of Australia.

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Hypertension. 1999;34:1112-1116
doi: 10.1161/01.HYP.34.5.1112

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