**AT\(_2\) Receptor Stimulation Enhances Antihypertensive Effect of AT\(_1\) 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 (AT\(_2\)) receptor in the regulation of blood pressure in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). We tested the hypothesis that AT\(_2\) receptor activation may contribute to the antihypertensive effects of angiotensin type 1 (AT\(_1\)) 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 AT\(_1\) receptor antagonist candesartan (0.01 or 0.1 mg/kg IV) alone, the AT\(_2\) receptor agonist CGP42112 (1 \(\mu\)g/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 AT\(_2\) 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 AT\(_2\) receptor antagonist PD123319 (50 \(\mu\)g/kg per minute) with the candesartan/CGP42112 combination. Collectively, these data suggest that in SHR, AT\(_2\) receptor activation can facilitate the initial depressor response caused by an AT\(_1\) receptor antagonist. (Hypertension. 1999;34:1112-1116.)

**Key Words:** receptors, angiotensin \(\beta\) vasodilation \(\beta\) angiotensin II \(\beta\) hypertension, arterial \(\beta\) 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 (AT\(_1\)) or angiotensin type 2 (AT\(_2\)). AT\(_1\) receptors are widely distributed throughout the body, including vascular smooth muscle, kidney, heart, and brain. AT\(_1\) receptors are responsible for mediating most of the known actions of Ang II, including vasoconstriction and aldosterone release.\(^{2}\) Not surprisingly, AT\(_1\) receptor antagonists have already proven to be clinically effective antihypertensive agents.\(^{3}\) However, the role of the AT\(_2\) receptor is less well defined.\(^{4}\) It is expressed in high levels in the developing fetus, which has led to the suggestion that the AT\(_2\) receptor is involved in growth and development.\(^{5-7}\)

Recently, a number of studies have implicated the AT\(_2\) receptor as having an opposing role to the AT\(_1\) receptor in certain experimental settings, including endothelial cell proliferation and neointimal formation. In both situations, the AT\(_1\) receptor causes stimulation, while the AT\(_2\) receptor mediates inhibition of the response.\(^{5,7,8}\) Similarly, we have recently reported that AT\(_2\) receptor blockade increased AT\(_1\) receptor–mediated contraction in the rat isolated uterine artery.\(^{9}\) Studies in transgenic mice have also suggested an inhibitory role of the AT\(_2\) receptor in blood pressure control since basal blood pressure and/or pressor sensitivity evoked by Ang II was increased in mice when the AT\(_2\) receptor gene had been disrupted.\(^{10}\)

Importantly, AT\(_1\) receptor antagonists are associated with a rise in plasma Ang II concentration due to the inhibition of the AT\(_1\) receptor–mediated negative feedback on renin release.\(^{3,6,12}\) Therefore, it has been suggested that, at therapeutic doses of AT\(_1\) receptor antagonists, endogenous Ang II may stimulate unopposed AT\(_2\) 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 AT\(_2\) receptor blockade.\(^{10,11,13,14}\)

Another approach has been to infuse Ang II in the presence of AT\(_1\) receptor blockade to stimulate AT\(_2\) 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, AT\(_2\) receptor stimulation increased aortic cyclic GMP content; however, any potential blood pressure changes may have been masked by...
direct vasoconstriction caused by infusion of a large dose of Ang II alone.15

Therefore, in the present study we determined whether selective AT2 receptor stimulation in vivo can alter blood pressure. In so doing, we tested the hypothesis that AT1 receptor–mediated vasodilatation may contribute to the antihypertensive effects of AT1 receptor antagonists. For this purpose, we used the highly specific AT2 receptor ligand CGP42112,16 which has been shown to act as an agonist in both studies using cells specifically expressing AT2 receptors17,18 and functional studies.7,9,19 Importantly, CGP42112 does not exert any cardiovascular effects at appropriate doses.20,21 Therefore, we determined the antihypertensive effect of the AT1 receptor antagonist candesartan,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 ~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 AT1 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.23,24 The AT1 receptor antagonist CGP42112 was given as an infusion at 1 μg/kg per minute for 4 hours, which was previously shown to be highly selective for AT1 receptors.20,21 The AT1 receptor antagonist PD123319 was given at 50 μg/kg per minute for 2 hours, on the basis of previous studies.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 (~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 μg/kg per minute) for 4 hours; and (3) a 4-hour infusion of CGP42112 alone (1 μg/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 μg/kg per minute) for 4 hours; and (3) candesartan (0.01 mg/kg IV) plus an infusion of CGP42112 (1 μg/kg per minute) for 4 hours and a 2-hour infusion of PD123319 (50 μg/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.

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

<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) + saline</td>
<td>114±2</td>
<td>307±16</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>122±4</td>
<td>333±17</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>CGP42112</td>
<td>111±4</td>
<td>320±8</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + saline</td>
<td>121±5</td>
<td>309±7</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>110±2</td>
<td>301±9</td>
</tr>
</tbody>
</table>

Values are mean±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 (0.01 mg/kg) + saline</td>
<td>155±7</td>
<td>310±10</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>169±7</td>
<td>324±12</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) + saline</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>Candesartan (0.01 mg/kg) + PD123319</td>
<td>170±6</td>
<td>341±17</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) + saline</td>
<td>179±8</td>
<td>313±13</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + CGP42112</td>
<td>168±4</td>
<td>308±7</td>
</tr>
<tr>
<td>Candesartan (0.01 mg/kg) + PD123319</td>
<td>166±6</td>
<td>310±14</td>
</tr>
</tbody>
</table>

Values are mean±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 μg/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).

**Figure 1.** Effects of the AT1 receptor antagonist candesartan (CV) (0.1 mg/kg IV) followed by a 4-hour infusion of either saline or CGP42112 (CGP) (1 μg/kg per minute) on MAP in WKY (n = 7). Values represent mean ± SEM. Four-hour infusions of saline and CGP42112 had no effect on MAP. **P < 0.01 for overall effect of CV vs baseline (ANOVA).**

**Figure 2.** Effects of a 10-fold lower dose of the AT1-receptor antagonist candesartan (CV) (0.01 mg/kg IV) followed by a 4-hour infusion of either saline or CGP42112 (CGP) (1 μg/kg per minute) on MAP in WKY (n = 6). Values represent mean ± SEM. *P < 0.05 for overall effect of CV+saline vs baseline (ANOVA).
The main finding of the present study is that AT2 receptor–mediated depressor responses were demonstrated, but only in the presence of AT1 receptor blockade. To our knowledge, this study represents the first in vivo demonstration of direct AT2 receptor–mediated vasodilatation in conscious SHR. Moreover, results from this study suggest that the AT1 receptor opposes the action of the AT2 receptor in blood pressure regulation, at least in SHR.

Previous studies using Ang II to stimulate AT2 receptors have been complicated by the direct (AT1 receptor–mediated) vasoconstrictor action of the peptide and/or inappropriate experimental designs, as noted. Therefore, in the present study CGP42112 was used as the AT2 receptor agonist because it is devoid of cardiovascular effects at up to 100 times the dose used here.20,21 The AT1 receptor antagonist candesartan is a potent, long-acting metabolite of candesartan cilexetil and causes prolonged inhibition of the vasoconstrictor effects of Ang II after a single intravenous dose.22–24

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.24,26 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 AT2 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,20,21 indicate that, at this dose, PD123319 exerts no cardiovascular effects per se. Further support for an inhibitory role of the AT2 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 AT2 receptor–mediated depressor component in conscious SHR, which contrasts with studies using AT1 receptor blockade combined with Ang II. Gohlke et al15 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 (≈20 mm Hg) 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.15 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 AT1 receptor blockade.13 However, this report has not been confirmed, and such covert depressor activity is not observed after injections of Ang II during AT1 receptor blockade in the conscious state.23,24,27 However, the present data are consistent with those of Munzenmaier and Greene,14 although that study was performed in salt-loaded rats.

Thus, our results support the emerging concept that an additional depressor effect due to AT2 receptor stimulation during AT1 receptor blockade may play a role in the beneficial effects of AT1 receptor antagonists.6,20 In this context, it has recently been reported that the cardiovascular effects of the AT1 receptor antagonist losartan were blocked by the coadministration of the AT2 receptor antagonist PD123319 in rats with heart failure28 or in rats with sodium depletion.25 The fact that AT2 receptor stimulation causes vasodilatation suggests that, in addition to the predominant AT1 receptor subtype, there are also AT2 receptors located in the vasculature. Indeed, early autoradiographic studies did in fact report that AT2 receptors account for ≈30% of Ang II receptors in aortic tissue.4 AT2 receptors have also been implicated in coronary endothelial cells5,8 and in skeletal muscle microvasculature.14 More recently, immunohistochemical studies have identified AT2 receptors in endothelium and vascular smooth muscle of large and small microvessels,29 and mRNA expression for both AT1 and AT2 receptors was demonstrated in aortic tissue.30 Moreover, there was enhanced expression of both receptor subtypes in SHR compared with WKY.30
Hypertension November 1999

1116

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.31,32 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 of candesartan by CGP42112 in the SHR suggests that AT2 receptors play a modulatory role in blood pressure regulation. Moreover, these data suggest that an AT2 receptor component should be considered as a potential complementary 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.30

Acknowledgments

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