AT₂ receptors contribute to acute blood pressure-lowering and vasodilator effects of AT₁ receptor antagonism in conscious normotensive but not hypertensive rats

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Duke, Lisa M., Roger G. Evans, and Robert E Widdop. AT₂ receptors contribute to acute blood pressure-lowering and vasodilator effects of AT₁ receptor antagonism in conscious normotensive but not hypertensive rats. Am J Physiol Heart Circ Physiol 288: H2289–H2297, 2005. First published December 22, 2004; doi:10.1152/ajpheart.01096.2004.—The aims of this study were to determine the contribution of the AT₂ receptor to the antihypertensive and regional vasodilatory effects of AT₁ receptor blockade in adult spontaneously hypertensive rats (SHR), 2-kidney, 1-clip hypertensive (2K1C) rats, and sham-operated normotensive rats. Several studies have provided evidence to support the notion that the AT₂ receptor may have opposing effects to those mediated by the AT₁ receptor. We therefore tested the hypothesis that the depressor and vasodilator effects of acute AT₁ receptor blockade are dependent on AT₂ receptor activation. Heart rate, mean arterial pressure, and regional hemodynamics were measured over a 4-day protocol in rats that received the following treatments in randomized order: saline vehicle, the AT₁ receptor antagonist candesartan (0.1 mg/kg iv bolus), the AT₂ receptor antagonist PD-123319 (50 μg·kg⁻¹·min⁻¹), or both antagonists. Intravenous candesartan reduced mean arterial pressure in all groups of rats, and this was accompanied by renal and mesenteric vasodilation. Neither saline nor PD-123319 significantly affected these variables. Concomitant PD-123319 administration partially reversed the depressor and mesenteric vasodilator effects of candesartan in sham-operated normotensive rats but not in SHR or 2K1C rats. These data indicate that the AT₂ receptor contributes to the blood pressure-lowering and mesenteric vasodilator effects of AT₁ receptor blockade in the acute setting in conscious normotensive but not hypertensive rats.

angiotensin receptors; candesartan; PD-123319; regional hemodynamics; hypertension; renal

ANGIOTENSIN II (ANG II), the main effector peptide of the renin angiotensin system (RAS), acts at two main receptor subtypes: the AT₁ receptor and the AT₂ receptor (7). The well-known ANG II actions such as regulation of blood pressure and water and electrolyte balance have been attributed mainly to the various signal transduction pathways activated by the AT₁ receptor (7). The role of the AT₂ receptor is less well defined. There is evidence that AT₂ receptor activation may oppose effects mediated by the AT₁ receptor. For example, AT₂ receptor activation appears to inhibit AT₁ receptor-mediated vasoconstriction and cell proliferation (3, 13, 16, 33, 44, 53).

AT₂ receptor-mediated vasodilation in vitro has been reported previously (2, 9, 11, 12, 29, 52), and these findings are further supported by in vivo data. For example, Barber et al. (3) found that in spontaneously hypertensive rats (SHR), the AT₂ receptor agonist CGP-42112 lowered blood pressure, but only in the presence of AT₁ receptor blockade, suggesting that AT₂ receptors play a modulatory role in the regulation of arterial pressure (3). This unmasked AT₂ receptor-mediated depressor effect of CGP-42112 was abolished by PD-123319. More recently, Li and Widdop (22) performed analogous experiments in which regional hemodynamics were measured and found that CGP-42112 evoked renal and mesenteric vasodilation in conscious SHR in the presence of AT₁ receptor blockade. Similarly, Carey et al. (5) reported that direct stimulation of the AT₂ receptor with CGP-42112 reduced arterial pressure in conscious rats and that this effect was blocked by PD-123319 (5). Collectively, these data suggest that in SHR, direct AT₂ receptor activation causes depressor and vasodilator responses, but only when these are unmasked by the presence of an AT₁ receptor antagonist (3, 22).

In vivo, AT₁ receptor antagonists induce increases in plasma ANG II concentration as a result of inhibition of the AT₁ receptor-mediated negative feedback on renin release (46). Therefore, it has been suggested that at therapeutic doses of AT₁ receptor antagonists, endogenous ANG II may stimulate AT₂ receptors, which in turn contributes to their depressor effects (6, 8). This hypothesis was recently extensively reviewed (51), and it was noted that there are very few studies examining blood pressure status in this context. In a 2-kidney, 1-wrap model of renovascular hypertension, activation of the RAS has been shown to increase arterial pressure through the AT₁ receptor (40). The antihypertensive effect of the AT₁ receptor antagonist losartan was prevented by AT₂ receptor blockade with PD-123319 and also by the use of the bradykinin B₂ receptor antagonist icatibant (40). In addition, acute studies from the same laboratories have documented that in conscious rats, sodium depletion caused an increase in renal interstitial fluid levels of cGMP that was blocked by PD-123319 (41, 42). Furthermore, valsartan reduced systolic blood pressure in both sodium-depleted and renovascular hypertensive rats, and this effect of valsartan was reversed by coinfusion of PD-123319 (43). Therefore, stimulation of the AT₂ receptor by endogenous ANG II appears to contribute to the antihypertensive action of AT₁ receptor antagonists, at least in the acute setting.

Collectively, the studies described above give the general impression that in experiments in which models of increased RAS activity (e.g., renovascular hypertension, low salt) are used, AT₂ receptor-mediated vasodilatation may contribute to the antihypertensive efficacy of AT₁ receptor antagonists. However, in these studies, only systolic blood pressure was measured, which does not necessarily clearly identify the hemodynamic changes that

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may have occurred. Therefore, in the present study we tested the acute effects of candesartan and PD-123319 (and their combination) on regional hemodynamics in conscious normotensive rats and in renin-dependent (2-kidney, 1-clip; 2K1C) and renin-independent (SHR) hypertensive rat models. In so doing, we have tested the hypothesis that AT2 receptor blockade would reverse the antihypertensive and regional vasodilatory effects of AT1 receptor blockade. We also hypothesized that this AT2 receptor component of AT1 receptor blockade would be manifest to a greater extent in 2K1C rats than in SHR or normotensive animals. The latter hypothesis was based on the likelihood that elevated circulating levels of ANG II in 2K1C rats (35) would cause marked AT2 receptor stimulation.

METHODS

Animals. Male Sprague-Dawley rats (Biological Research Laboratories, Baker Heart Institute, Victoria, Australia; n = 14) and SHR (Biological Research Laboratories, Austin Repatriation Medical Centre, Victoria, Australia; n = 10) were used. Sprague-Dawley rats were used to establish renovascular hypertension, and SHR rats were used as an established non-renin-dependent model of hypertension. Procedures were approved by the Monash University Department of Pharmacology Animal Ethics Committee and accorded with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Establishment of hypertension. To establish 2K1C hypertension, we anesthetized Sprague-Dawley rats ~4 wk of age (100–150 g) with isoflurane (1–3%; Abbott Australasia, Kurnell, Australia), and a U-shaped silver clip (0.2-mm ID) was placed on the right renal artery as described previously (23). A sham control group of rats received a similar surgical intervention except that the silver clip was not inserted. Tail-cuff systolic blood pressure (SBP) was measured in both groups at weekly intervals (NIBP controller; ADInstruments). The two wire leads for each probe were joined to a six-port connector (Microtech, Boothwyn, PA) under a microscope (Olympus, Tokyo, Japan). Two catheters (polyethylene tubing, 0.28-mm ID, 0.61-mm OD; Bicoorp Australia, Huntingdale, Victoria, Australia) were inserted ~2 cm into the jugular vein for intravenous administration of drugs or saline, and a catheter (polyethylene tubing, 0.58-mm ID, 0.96-mm OD; Bicoorp Australia) was inserted ~2 cm into the right carotid artery for measurement of arterial blood pressure and heart rate (HR). The catheters were then tunneled subcutaneously to emerge at the same point as the Doppler probes (posterior neck region) and were inserted through a flexible spring that was secured along with the six-port microconnector to a custom-made harness worn by the rat. All animals were then allowed a further 24- to 48-h recovery period, during which time they had free access to food and water. Arterial catheters were kept patent by a constant infusion of heparinized saline (15 IU/ml; David Bull Laboratories, Mulgrave, Victoria, Australia) at 1 ml·kg⁻¹·h⁻¹ at all times other than the acute experimental protocols.

Experimental protocol. All experiments were conducted in conscious, freely moving rats. On the day of experimentation, continuous recordings were made of phasic intra-arterial blood pressure, HR, and mean and phasic renal, mesenteric, and hindquarter blood flow. In all rats, baseline levels of cardiovascular variables were recorded initially for at least 60 min before the administration of any drug. All rats were studied over a 4-day protocol. In SHR (n = 10), sham (n = 7), and 2K1C rats (n = 7), we tested the effects of I) saline (0.35 ml/h iv), 2) AT1 receptor blockade (candesartan; 0.1 mg/kg iv bolus plus saline infusion), 3) AT2 receptor blockade (PD-123319; 50 µg·kg⁻¹·min⁻¹ iv), or 4) combined AT1 and AT2 receptor blockade, each on a different day. The PD-123319 infusion was commenced 60 min before the administration of candesartan. These treatments were given in a random order. Responses to an intravenous bolus of ANG II (5–25 ng/kg) were tested at baseline and at 2, 4, and 6 h after the vehicle and/or antagonist treatments. All doses were based on previous studies in our laboratory (3, 21–23).

At the time of experimentation, all variables were displayed on a MacLab8 system (ADInstruments) interfaced with a Macintosh computer (23). Doppler shift is an index of blood flow, whereas regional vascular conductances were calculated by dividing the appropriate mean Doppler shift signal by mean arterial pressure (MAP) (23, 50).

Statistical analysis. Data are expressed as means ± SE and were subjected to analysis of variance (ANOVA) with the computer software SYSTAT (version 9; SPSS, Chicago, IL). Changes in hemodynamic variables from pretreatment baseline, on any given treatment day, were analyzed using repeated-measures ANOVA. For comparisons between different experimental subjects, Student’s unpaired t-test was used to determine whether SBP changed after sham or 2K1C surgery. ANOVA was used to determine, within each group of rats, whether baseline levels of hemodynamic variables differed before any antagonist treatment was administered. Partitioned ANOVA also allowed us to test whether baseline levels of hemodynamic variables differed in the three groups of rats. Repeated-measures ANOVA (26) was used to determine the effects and interactions of the antagonist treatments. To protect against the increased risk of a false positive inference due to compound asymmetry, P values from within-subject factors were conservatively adjusted using the Greenhouse-Geisser method (26). Statistical significance was accepted as P ≤ 0.05.

RESULTS

Tail-cuff SBP in conscious sham-operated and 2K1C rats. SBP of sham rats remained constant (118 ± 4 mmHg) over the 3- to 4-wk measurement period. In contrast, SBP of 2K1C rats rose significantly to 189 ± 3 mmHg (P < 0.001) 3 wk after surgery (Fig. 1). AJP-Heart Circ Physiol • VOL. 288 • MAY 2005 • www.ajpheart.org

Fig. 1. Tail-cuff systolic blood pressure (SBP) was recorded before (week 0) and 3 wk after surgery (clipping) in 2-kidney, 1-clip (2K1C) rats and sham-operated rats. Bars show means ± SE (n = 7 rats/group). **P < 0.001 vs. respective control (paired t-test).

Resting hemodynamics. Baseline levels of MAP, HR, and regional hemodynamic variables were similar to those ob-
Table 1. Resting baseline hemodynamic variables for all groups before antagonist treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Cand + Saline</th>
<th>Cand + PD</th>
<th>PD + Saline</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Normotensive Rats</td>
<td>362±16</td>
<td>367±19</td>
<td>370±29</td>
<td>397±26</td>
<td>0.70</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>113±4</td>
<td>119±2</td>
<td>110±5</td>
<td>109±55</td>
<td>0.28</td>
</tr>
<tr>
<td>Renal flow, kHz</td>
<td>5.1±1.3</td>
<td>4.2±1.0</td>
<td>4.7±1.3</td>
<td>4.3±1.1</td>
<td>0.94</td>
</tr>
<tr>
<td>Mesenteric flow, kHz</td>
<td>4.7±1.0</td>
<td>4.5±0.9</td>
<td>4.4±0.7</td>
<td>4.8±0.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Hindquarter flow, kHz</td>
<td>2.9±1.1</td>
<td>3.6±0.8</td>
<td>2.6±0.3</td>
<td>3.2±0.4</td>
<td>0.80</td>
</tr>
<tr>
<td>Ren cond, kHz/mmHg × 10³</td>
<td>45.5±12.2</td>
<td>35±8.1</td>
<td>42.1±11.1</td>
<td>39.7±11.8</td>
<td>0.91</td>
</tr>
<tr>
<td>Mes cond, kHz/mmHg × 10³</td>
<td>42.9±9.7</td>
<td>37.5±7.0</td>
<td>40.8±7.1</td>
<td>44±6.6</td>
<td>0.91</td>
</tr>
<tr>
<td>HQ cond, kHz/mmHg × 10³</td>
<td>26.1±10.3</td>
<td>30.1±6.5</td>
<td>23.8±0.0</td>
<td>29.0±3.6</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Table 2. Resting hemodynamic variables, recorded on separate days, before treatment in sham-operated normotensive rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Cand + Saline</th>
<th>Cand + PD</th>
<th>PD + Saline</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Normotensive Rats</td>
<td>404±12</td>
<td>390±14</td>
<td>397±12</td>
<td>399±9</td>
<td>0.66</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>149±8</td>
<td>142±9</td>
<td>145±9</td>
<td>139±11</td>
<td>0.85</td>
</tr>
<tr>
<td>Renal flow, kHz</td>
<td>6.7±2.0</td>
<td>5.3±1.4</td>
<td>5.3±1.7</td>
<td>4.6±2.1</td>
<td>0.94</td>
</tr>
<tr>
<td>Mesenteric flow, kHz</td>
<td>3.3±0.6</td>
<td>4.2±0.6</td>
<td>4.5±0.9</td>
<td>5.4±0.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Hindquarter flow, kHz</td>
<td>5.0±1.2</td>
<td>5.4±1.3</td>
<td>4.9±0.8</td>
<td>5.0±1.0</td>
<td>0.99</td>
</tr>
<tr>
<td>Ren cond, kHz/mmHg × 10³</td>
<td>45.3±13.2</td>
<td>37.8±9.6</td>
<td>34.5±9.5</td>
<td>41.6±14.6</td>
<td>0.80</td>
</tr>
<tr>
<td>Mes cond, kHz/mmHg × 10³</td>
<td>23.1±4.8</td>
<td>29.4±3.5</td>
<td>32.1±7.2</td>
<td>39.1±5.3</td>
<td>0.17</td>
</tr>
<tr>
<td>HQ cond, kHz/mmHg × 10³</td>
<td>31.9±6.2</td>
<td>38.8±9.8</td>
<td>33.9±4.7</td>
<td>35.5±6.9</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Table 3. Resting hemodynamic variables, recorded on separate days, before treatment in 2K1C hypertensive rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Cand + Saline</th>
<th>Cand + PD</th>
<th>PD + Saline</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Normotensive Rats</td>
<td>328±13</td>
<td>309±10</td>
<td>336±12</td>
<td>338±22</td>
<td>0.43</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>167±7</td>
<td>160±7</td>
<td>162±8</td>
<td>161±7</td>
<td>0.92</td>
</tr>
<tr>
<td>Renal flow, kHz</td>
<td>3.9±0.3</td>
<td>3.2±0.5</td>
<td>2.7±0.4</td>
<td>3.5±0.6</td>
<td>0.31</td>
</tr>
<tr>
<td>Mesenteric flow, kHz</td>
<td>4.0±0.6</td>
<td>4.5±0.8</td>
<td>5.1±1.1</td>
<td>4.3±1.1</td>
<td>0.85</td>
</tr>
<tr>
<td>Hindquarter flow, kHz</td>
<td>1.6±0.2</td>
<td>1.5±0.2</td>
<td>1.9±0.2</td>
<td>2.4±0.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Ren cond, kHz/mmHg × 10³</td>
<td>24.2±2.2</td>
<td>21.5±4.2</td>
<td>17.2±2.7</td>
<td>23.8±5.6</td>
<td>0.57</td>
</tr>
<tr>
<td>Mes cond, kHz/mmHg × 10³</td>
<td>24.4±4.0</td>
<td>27.9±5.1</td>
<td>32.4±7.4</td>
<td>27.4±6.7</td>
<td>0.80</td>
</tr>
<tr>
<td>HQ cond, kHz/mmHg × 10³</td>
<td>10.1±1.7</td>
<td>9.1±1.7</td>
<td>12.3±1.6</td>
<td>14.7±3.2</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table 4. Resting hemodynamic variables, recorded on separate days, before treatment in SHR

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Cand + Saline</th>
<th>Cand + PD</th>
<th>Saline + PD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Normotensive Rats</td>
<td>328±13</td>
<td>309±10</td>
<td>336±12</td>
<td>338±22</td>
<td>0.43</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>167±7</td>
<td>160±7</td>
<td>162±8</td>
<td>161±7</td>
<td>0.92</td>
</tr>
<tr>
<td>Renal flow, kHz</td>
<td>3.9±0.3</td>
<td>3.2±0.5</td>
<td>2.7±0.4</td>
<td>3.5±0.6</td>
<td>0.31</td>
</tr>
<tr>
<td>Mesenteric flow, kHz</td>
<td>4.0±0.6</td>
<td>4.5±0.8</td>
<td>5.1±1.1</td>
<td>4.3±1.1</td>
<td>0.85</td>
</tr>
<tr>
<td>Hindquarter flow, kHz</td>
<td>1.6±0.2</td>
<td>1.5±0.2</td>
<td>1.9±0.2</td>
<td>2.4±0.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Ren cond, kHz/mmHg × 10³</td>
<td>24.2±2.2</td>
<td>21.5±4.2</td>
<td>17.2±2.7</td>
<td>23.8±5.6</td>
<td>0.57</td>
</tr>
<tr>
<td>Mes cond, kHz/mmHg × 10³</td>
<td>24.4±4.0</td>
<td>27.9±5.1</td>
<td>32.4±7.4</td>
<td>27.4±6.7</td>
<td>0.80</td>
</tr>
<tr>
<td>HQ cond, kHz/mmHg × 10³</td>
<td>10.1±1.7</td>
<td>9.1±1.7</td>
<td>12.3±1.6</td>
<td>14.7±3.2</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Data are means ± SE of average values of each variable during the 60-min control period before antagonist treatments were administered. HR, heart rate; MAP, mean arterial pressure; Ren cond, renal conductance; Mes cond, mesenteric conductance; HQ cond, hindquarter conductance. P values represent the outcomes of one-way analysis of variance (Bonferroni’s multiple comparison test), testing whether the baseline hemodynamic variables differed between groups. *P < 0.001, sham vs. 2K1C. #P < 0.05, ¥P < 0.01 and ¥¥P < 0.001, sham vs. spontaneously hypertensive rats (SHR). †P < 0.05, ‡P < 0.01 and §§P < 0.001, SHR vs. 2-kidney, 1-clip (2K1C) rats.
hindquarter flows and conductances were indistinguishable from those after candesartan alone. However, coinfusion of PD-123319 abolished the depressor and mesenteric vasodilatory (increased mesenteric flow and conductance) effect of candesartan (Fig. 2).

2K1C hypertensive rats. In 2K1C rats, candesartan had a minimal effect on HR but caused an immediate, marked decrease in MAP (maximum: 23 ± 4 mmHg) (Fig. 3), which was accompanied by renal and mesenteric vasodilatation (47 ± 9 and 22 ± 10% increases in conductances, respectively). Candesartan had no significant effect on hindquarter flow or conductance (Fig. 3). HR, MAP, and renal, mesenteric, and hindquarter flows and conductances were not significantly affected by saline or PD-123319 treatment (Fig. 3). When PD-123319 was coinfused with candesartan, HR, MAP, and regional flows and conductances were indistinguishable from those after candesartan alone (Fig. 3).

SHR. In SHR, candesartan administration was followed by a slight, transient tachycardia (Fig. 4). Candesartan caused an immediate, marked decrease in MAP (maximum: 27 ± 5 mmHg) (Fig. 4), which was accompanied by renal vasodilatation (28 ± 4 and 49 ± 8% increases in renal flow and conductance) and mesenteric vasodilatation (21 ± 11% increase in mesenteric conductance). Candesartan had no significant effect on hindquarter flow or conductance (Fig. 4). HR, MAP, and renal and hindquarter flows and conductances were not significantly affected by saline or PD-123319 treatment (Fig. 4). However, mesenteric flow was decreased after saline (21 ± 7%) and PD-123319 (25 ± 8%) treatment (data not shown), although the reductions in mesenteric conductance failed to reach significance (Fig. 4). When PD-123319 was coinfused with candesartan, HR, MAP, and regional flows and conductances were indistinguishable from those after candesartan alone (Fig. 4).

Responses to ANG II. In each group, responses to an intravenous bolus of ANG II (5–25 ng/kg) were measured before and after drug administration to determine the level of AT1 blockade induced by candesartan (Figs. 5–7). In all sham normotensive and SHR rats, a 5 ng/kg dose of ANG II was sufficient to induce a pressor response of ~20 mmHg. However, in three of the seven 2K1C rats, a 25 ng/kg dose of ANG II was required to induce a pressor response of ~20 mmHg. These pressor responses to ANG II were accompanied by marked renal and mesenteric vasoconstriction (decreased conductances) and variable effects on the hindquarters (Figs. 5–7). The vasoconstrictor effects of ANG II were markedly reduced (usually by 70–90%) by candesartan alone and in combination with saline or PD-123319 treatment (data not shown), whereas the regional hemodynamic effect of ANG II was reproducible over 6 h in the rats treated with saline.

DISCUSSION
In the present study, we examined whether the depressor and regional vasodilator effects of acute AT1 receptor blockade could be reversed with acute AT2 receptor antagonism. Adult male SHR were used as a model of genetic hypertension, 2K1C rats were

Fig. 2. Effects of AT1 and AT2 receptor blockade on systemic and regional hemodynamics in normotensive rats. PD-123319 (PD; 50 μg·kg⁻¹·min⁻¹ iv) or saline commenced 60 min before candesartan (Cand; 0.1 mg/kg iv) and continued for 7 h of the experiment. HR, heart rate; MAP, mean arterial pressure; Ren Cond, renal conductance; Mes Cond, mesenteric conductance; HQ Cond, hindquarter conductance. Symbols and error bars represent means ± SE, respectively, of the changes in HR, MAP, and renal, mesenteric, and hindquarter conductances during the experimental period. *P < 0.05 and ***P < 0.001, overall effect vs. baseline (ANOVA). #P < 0.05, treatment-time interaction between candesartan and PD-123319 (ANOVA).
Fig. 3. Effects of AT₁ and AT₂ receptor blockade on systemic and regional hemodynamics in 2K1C rats. PD-123319 (50 μg·kg⁻¹·min⁻¹ iv) or saline commenced 60 min before candesartan (0.1 mg/kg iv) and continued for 7 h of the experiment. Symbols and error bars represent means ± SE, respectively, of the changes in HR, MAP, and renal, mesenteric, and hindquarter conductances during the experimental period. *P < 0.05 and ***P < 0.001, overall effect vs. baseline (ANOVA).

Fig. 4. Effects of AT₁ and AT₂ receptor blockade on systemic and regional hemodynamics in spontaneously hypertensive rats (SHR). PD-123319 (50 μg·kg⁻¹·min⁻¹ iv) or saline commenced 60 min before candesartan (0.1 mg/kg iv) and continued for 7 h of the experiment. Symbols and error bars represent means ± SE, respectively, of the changes in HR, MAP, and renal, mesenteric and hindquarter conductances during the experimental period. **P < 0.01 for overall effect vs. baseline (ANOVA).
Fig. 5. Systemic and regional hemodynamic responses to ANG II in sham-operated normotensive rats. Bolus doses of ANG II (5 ng/kg iv) were given before (0 h) and at 2, 4, and 6 h after treatment with saline, candesartan (0.1 mg/kg iv bolus), PD-123319 (50 μg·kg⁻¹·min⁻¹ iv), or candesartan + PD-123319. P values represent the outcomes of Dunnett’s test comparing responses after the treatments to the respective control responses: *P ≤ 0.05 and ***P ≤ 0.001 vs. respective control.

Fig. 6. Systemic and regional hemodynamic responses to ANG II in 2K1C rats. Bolus doses of ANG II (5–25 ng/kg iv) were given before (0 h) and at 2, 4, and 6 h after treatment with saline, candesartan (0.1 mg/kg iv bolus), PD-123319 (50 μg·kg⁻¹·min⁻¹ iv), or candesartan + PD-123319. P values represent the outcomes of Dunnett’s test comparing responses after the treatments to the respective control responses: *P ≤ 0.05 and ***P ≤ 0.001 vs. respective control.
used as a renin-dependent model of hypertension, and sham-operated rats were used as their normotensive controls. To our knowledge, this is the first study investigating the potential role of AT2 receptors in regional hemodynamic responses to acute AT1 receptor blockade in a range of conscious rat models.

The major new finding from the present study was that the blood pressure-lowering effect of AT1 receptor antagonism was reversed by PD-123319 in normotensive rats but not in SHR or 2K1C hypertensive rats. We had hypothesized that AT2 receptor-mediated vasodilatation might contribute to the hemodynamic profile of AT1 receptor antagonists and that the contribution was likely to be greater in hypertensive rats, particularly 2K1C rats, because of elevated ANG II levels (35). We now reject this hypothesis, because PD-123319 did not reverse the antihypertensive or vasodilator responses to candesartan in 2K1C or SHR rats.

In the present study, AT1 receptor blockade caused reductions in blood pressure in all rat models. This was accompanied by marked renal vasodilatation in the hypertensive rat models but by an inconsistent effect in this vascular bed in normotensive rats. This observation is consistent with the findings of previous studies in our laboratory (21, 23), in which candesartan evoked more marked renal vasodilatation in hypertensive than normotensive rats. Candesartan also caused mesenteric dilation in both normotensive and hypertensive rats, with little effect in the hindquarter circulation. Again, these findings are consistent with those of previous studies in our laboratory (21–23), in which mesenteric vasodilatation, induced by candesartan, was more marked than vasodilatation in the hindquarter vascular bed. However, in hypertensive rats, PD-123319 failed to alter the antihypertensive and renal vasodilator responses to candesartan, indicating no role of the AT2 receptor in mediating vasodilatation initiated by AT1 receptor blockade under these experimental conditions. Similarly, Nakamura et al. (34) reported minimal reversal (~8 mmHg) by PD-123319 of the antihypertensive effect (~46 mmHg) of losartan in SHR, an effect that is not inconsistent with our observations in SHR. The dose of PD-123319 used in the present study was shown previously to block the vasodilator effects of the AT2 receptor agonist CGP-42112 in vivo (3, 22).

The lack of reversal by PD-123319 of the depressor response of AT1 receptor blockade is in marked contrast to the results reported by Siragy and colleagues (40, 43), who found that losartan or valsartan reduced SBP in both sodium-depleted and renovascular hypertensive rats, and this antihypertensive effect was reversed by coinfusion of PD-123319. The reasons for the discrepancies between the current and previous studies are not immediately obvious but possibly could relate to different models and experimental conditions. AT2 receptor expression varies with age, being highly expressed in fetal tissue but immediately declining after birth to a lower level of expression in vasculature that exists in adulthood (see Ref. 51). However, age is not likely to be a factor in the current study, because male normotensive and 2K1C rats were the same age (10 wk), which was not dissimilar to the age of rats used by Siragy and colleagues (40, 43). However, these authors used a renal wrap model of renovascular hypertension or sodium depletion and measured noninvasive SBP, which differs from invasive blood pressure measurements in the present study. They also measured intrarenal hemodynamics in the anesthetized state and found that PD-123319 prevented the increase in renal cortical
blood flow induced by losartan administration, although the effect on total renal blood flow was not reported (40). On the other hand, we (10) recently reported that PD-123319 did not affect responses of renal or cortical blood flow to candesartan.

In the present study, AT2 receptor-mediated mesenteric vasodilatation was implicated in normotensive rats, because administration of the AT2 receptor antagonist reversed the AT1 receptor antagonist-induced increase in mesenteric blood flow. Thus acute AT2 receptor activation appears to exert vasodilator effects in the mesenteric circulation and contribute to the blood pressure-lowering efficacy of AT1 receptor antagonists in normotensive but not hypertensive rats, at least in the acute setting. These data are consistent with the findings of Matrougui and colleagues (28, 29), who reported that AT2 receptors mediate relaxation of isolated mesenteric arteries of Wistar-Kyoto (WKY) but not SHR rats. This lack of AT2 receptor-mediated vasodilatation in SHR was postulated to be due to impaired nitric oxide- and prostaglandin-mediated responses to flow in resistance arteries of hypertensive rats (30). Given that AT2 receptors stimulate vasodilatation by an autocrine cascade including bradykinin, nitric oxide, and cGMP, the lack of effect of AT2 receptor blockade in hypertensive rats in the present study also may be due to impaired signaling pathways, as is known to occur with nitric oxide in the hypertensive state (1, 20, 38), and most likely involves increased oxidative stress reducing nitric oxide bioavailability (49). These functional data do not exactly correspond with studies in which vascular cGMP production is measured, since Gollik et al. (14) first reported that AT2 receptor stimulation increased cGMP in aortas obtained from SHR. On the other hand, AT2 receptor stimulation in aortas obtained from normotensive rats has been reported to exert no effect (37) or increased cGMP production (17).

Furthermore, the situation is much more complex in vivo, because direct AT2 receptor stimulation by exogenous CGP-42112 does, in fact, cause vasodilatation in SHR but not WKY rats, because AT2 receptor-mediated vasodilatation occurred in response to infusion with CGP-42112, but only in the presence of AT1 receptor blockade (3, 22). Therefore, there appears to be a difference between the effects of acute exogenous AT2 receptor stimulation during AT1 receptor blockade and the effects of acute exogenous AT2 receptor stimulation with the synthetic peptide CGP-42112.

In the present study, bolus injections of ANG II were obtained to determine the level of AT1 receptor blockade induced by candesartan. ANG II caused marked increases in MAP that were associated with decreases in renal and mesenteric conductance and variable responses of hindquarter conductance, as previously reported (21–23, 50). However, in 2K1C rats, pressor responses to ANG II were less than those in SHR or normotensive rats, with higher doses of ANG II sometimes required to match pressor responses. The most likely explanation for this is that the vascular lature is less sensitive to ANG II, because circulating (36) and intrarenal levels (15) of ANG II are elevated in rats with 2K1C hypertension. In any case, candesartan alone or in combination with PD-123319 markedly reduced ANG II-mediated vasoconstriction. Indeed, the effects of candesartan were very prolonged, similar to those we have noted previously, even from a single intravenous dose (21–23, 50). In vitro, PD-123319 has been shown to potentiate contractile responses to ANG II (2, 16, 31, 53), suggesting that the activation of the AT2 receptor is linked to a vasodilator mechanism that opposes vasoconstriction induced by AT1 receptor activation. Potentiation of ANG II pressor responses in PD-123319-treated animals has occasionally been seen in vivo (32), although usually this is not seen (22, 27), as was the case in the present study. Furthermore, there was little evidence of AT2 receptor-mediated vasodilatation evoked by exogenous ANG II (in the presence of candesartan), which presumably indicates that bolus administration of ANG II was less effective than longer infusions designed to stimulate AT2 receptors (3, 22).

Collectively, the results of the present study indicate that acute, AT2 receptor activation contributes to the acute hemodynamic effects of AT1 receptor blockade in conscious normotensive, but not hypertensive, rats. However, this is clearly not the case with respect to cardiovascular structural remodeling, particularly under certain pathological conditions in which it is known that AT2 receptors are upregulated (4, 18, 25, 39, 47). In this context, AT2 receptor stimulation has an important role in the cardiovascular protective effects seen with chronic treatment with AT1 receptor antagonists, because chronic administration of PD-123319 reversed the decreases in cardiac fibrosis and vascular remodeling observed with AT1 receptor antagonists in rat models of hypertension (SHR), heart failure, and senescence (19, 24, 45, 48). Therefore, the results of these studies indicate a role for the AT1 receptor in mediating the beneficial structural effects of AT1 receptor antagonist therapy, although there appears to be minimal influence of the AT2 receptor on sartan-mediated changes in arterial pressure under both acute (present study) and chronic (19, 24, 45, 48) pathological conditions. However, a role for an AT2 receptor-mediated contribution to the acute hemodynamic effect of sartan compounds in normotensive rats was established.

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REFERENCES

HEMODYNAMIC ROLE OF AT2 RECEPTORS DURING ACUTE AT1 RECEPTOR BLOCKADE


