Disparate Roles of AT₂ Receptors in the Renal Cortical and Medullary Circulations of Anesthetized Rabbits

Lisa M. Duke, Gabriela A. Eppel, Robert E. Widdop, Roger G. Evans

Abstract—The contributions of angiotensin II type 1 (AT₁) and type 2 (AT₂) receptors to the control of regional kidney blood flow were determined in pentobarbital-anesthetized rabbits. Intravenous candesartan (AT₁ antagonist; 10 μg/kg plus 10 μg · kg⁻¹ · h⁻¹) reduced mean arterial pressure (12%) and increased total renal blood flow (29%) and cortical laser-Doppler flux (18%) but not medullary laser-Doppler flux. Neither intravenous PD123319 (AT₂ antagonist; 1 mg/kg plus 1 mg · kg⁻¹ · h⁻¹) nor saline vehicle significantly affected these variables, and the responses to candesartan plus PD123319 were indistinguishable from those of candesartan alone. In vehicle-treated rabbits, renal-arterial infusions of angiotensin II (1 to 25 ng · kg⁻¹ · min⁻¹) and angiotensin III (5 to 125 ng · kg⁻¹ · min⁻¹) dose-dependently reduced renal blood flow (up to 51%) and cortical laser-Doppler flux (up to 50%) but did not significantly affect medullary laser-Doppler flux or arterial pressure. Angiotensin(1–7) (20 to 500 ng · kg⁻¹ · min⁻¹) had similar effects but of lesser magnitude. CGP42112A (20 to 500 ng · kg⁻¹ · min⁻¹) did not significantly affect these variables. After PD123319 administration, angiotensin II and angiotensin III dose-dependently increased medullary laser-Doppler flux (up to 84%), and reductions in renal blood flow in response to angiotensin II were enhanced. Candesartan abolished renal hemodynamic responses to the angiotensin peptides, even when given in combination with PD123319. We conclude that AT₂ receptor activation counteracts AT₁-mediated vasoconstriction in the renal cortex but also counteracts AT₁-mediated vasodilatation in vascular elements controlling medullary perfusion. These mechanisms might have an important effect on the control of medullary perfusion under conditions of activation of the renin-angiotensin system.

Key Words: receptors, angiotensin • kidney • laser-Doppler flowmetry • rabbits • renal circulation

The medullary circulation contributes to the control of mean arterial pressure (MAP) and body fluid homeostasis. However, the roles of angiotensin II type 1 (AT₁) and type 2 (AT₂) receptors in regulating regional kidney perfusion remain unclear. In rats and rabbits, infusions of angiotensin II reduce total renal blood flow (RBF) and cortical blood flow but have a lesser effect on medullary blood flow (MBF). Angiotensin II can even increase MBF, especially when administered as a bolus. Nitric oxide synthase and/or cyclooxygenase blockade can enhance angiotensin II–induced reductions in MBF and abolish angiotensin II–induced increases in MBF, both of which are chiefly AT₁ mediated. However, the contributions of AT₂ receptors to these effects have received little attention, even though they are expressed in vessels that might contribute to MBF control (eg, afferent arterioles and vasa recta).

AT₂-mediated counterregulatory vasodilatation can oppose AT₁-mediated vasoconstriction. For example, in rat renal-wrap hypertension, AT₂ receptor stimulation blunts AT₁-mediated hypertension. Furthermore, in spontaneously hypertensive rats, the antihypertensive effect of candesartan was potentiated by coinfusion of the AT₂ agonist CGP42112.

How AT₂ receptors influence renal excretory function remains less clear. Nevertheless, despite some data to the contrary, most data support the notion that AT₂ receptor activation promotes natriuresis, so opposing AT₁-mediated antinatriuresis.

We therefore hypothesized that AT₂-mediated vasodilatation might contribute both to the relative insensitivity of the medullary microcirculation to angiotensin II–induced vasoconstriction and to increases in MBF in response to boluses of angiotensin peptides. Consistent with this notion, AT₂ receptor activation opposes AT₁-mediated vasoconstriction and endothelial calcium signaling in isolated, descending vasa recta in vitro. Therefore, we tested the effects of candesartan and PD123319 on the responses of regional kidney perfusion and renal excretory function to the renal-arterial administration (infusion and bolus) of angiotensin peptides in anesthetized rabbits. We tested the responses to angiotensin II itself, angiotensin III (moderate selectivity for AT₂ receptors), the highly selective AT₂ agonist CGP42112A, and angiotensin(1–7), because the latter has both a high affinity for AT₂ receptors and vasodilator effects that are possibly mediated independently of AT₁ and AT₂ receptors.
Methods

Animals
Male New Zealand White rabbits (Monash University Central Animal Services, Victoria, Australia; 2.87±0.05 kg) were used and were humanely killed after the experiments with an overdose of sodium pentobarbital (300 mg IV). Procedures were approved by the Monash University Department of Physiology Animal Ethics Committee.

Surgical Procedures
These have been described previously.10,11,24,25 Anesthesia was induced with sodium pentobarbital (90 to 150 mg plus 30 to 50 mg/h). Artificial ventilation and infusion of maintenance solutions then continued for the entire experiment.25 Both kidneys were denervated and both ureters catheterized.24,25 The left kidney was placed in a stable cup, and a renal artery was side-branch catheterized. Left kidney RBF, and cortical blood flow and MBF were monitored by transit-time ultrasound flowmetry and laser-Doppler flowmetry, respectively.10,11,26 Experiments commenced 60 to 90 minutes after surgery was completed.

Experimental Protocol
After a 10-minute control period, rabbits (n=6 per group) were treated with (1) the AT1 antagonist candesartan (10 μg/kg IV plus 10 μg · kg⁻¹ · h⁻¹ IV; Astra Zeneca), (2) the AT1 antagonist PD123319 (1 mg/kg plus 1 mg · kg⁻¹ · h⁻¹ IV; synthesized as previously described12), (3) both antagonists, or (4) saline vehicle (1 mL/kg IV plus 1 mL · kg⁻¹ · h⁻¹ 154 mmol/L NaCl). These infusions continued for the entire experiment. Urine produced by both kidneys was collected during the control period and for 20 to 30 minutes after the antagonist treatments commenced. Arterial blood (0.5 mL) was collected at the midpoint of each clearance period.

Thirty minutes after commencing antagonist infusions, renal-arterial infusions of angiotensin II (0, 1, 5, and 25 ng · kg⁻¹ · min⁻¹, Auspep), CGP42112A (0, 20, 100, and 500 ng · kg⁻¹ · min⁻¹, Bachem), angiotensin III (0, 5, 25, and 125 ng · kg⁻¹ · min⁻¹, Auspep), and angiotensin (1–7) (0, 20, 100, and 500 ng · kg⁻¹ · min⁻¹, Auspep) were given in random order. Each dose was administered over 12 minutes, and urine and blood samples were taken during the final 6 minutes of each period. A recovery period of at least 12 minutes followed the final dose of each agonist. After the peptide infusions, a bolus dose of each peptide (angiotensin II, 50 ng/kg; CGP42112A, 1 μg/kg; angiotensin III, 250 ng/kg; and angiotensin (1–7), 1 μg/kg) was administered to the renal artery at 5-minute intervals in random order.

Measurements
A data acquisition system26 provided 2-second means of MAP (in mm Hg), heart rate (HR, beats per minute [bpm]), RBF (mL/min), and cortical and medullary laser-Doppler flux (CLDF and MLDF, units). Hematocrit, glomerular filtration rate (GFR; [¹²⁵I]iodohippurin clearance), effective renal blood flow (ERBF; [¹⁴C]p-aminohippuric acid clearance corrected for hematocrit), and urine and plasma sodium and potassium concentrations were determined as previously described.24 RBF and renal excretory variables were normalized by dry kidney weight (1.94±0.06 and 1.90±0.05 g for left and right kidneys, respectively).

Statistical Methods
Data are expressed as mean±SEM. Values of P<0.05 were considered statistically significant. ANOVA and, where appropriate, repeated-measures ANOVA28 were used to evaluate the effects and interactions of the antagonist treatments.

Results
Baseline Measurements
Baseline levels of MAP, HR, RBF, CLDF, and MLDF, when averaged for the 24 rabbits, were 70±1 mm Hg, 262±3 bpm, 15.0±0.8 mL · min⁻¹ · g⁻¹, 346±15 U, and 56±7 U, respectively. Renal clearance measurements were similar in the left and right kidneys, with the following average values: ERBF, 15.0±2.0 mL · min⁻¹ · g⁻¹; GFR, 1.6±0.2 mL · min⁻¹ · g⁻¹; filtration fraction, 0.20±0.02; urine flow, 0.21±0.03 mL · min⁻¹ · g⁻¹; sodium excretion, 25±5 μmol · min⁻¹ · g⁻¹; potassium excretion, 1.7±0.2 μmol · min⁻¹ · g⁻¹; fractional volume excretion, 0.19±0.03; fractional sodium excretion, 0.14±0.03; and fractional potassium excretion, 0.50±0.10. None of the measured variables differed according to treatment (vehicle, candesartan, PD123319, or candesartan+PD123319) that was to follow (P_group≥0.20).

Responses to Candesartan and PD123319
MAP, HR, RBF, CLDF, and MLDF were not significantly affected by vehicle or PD123319 treatment. In contrast, after candesartan, MAP was decreased by 12±2%, accompanied by increases in RBF (29±5%) and CLDF (18±2%). Candesartan did not significantly affect MLDF (+6±12% change) or HR. In rabbits in which PD123319 was coinfused with candesartan, responses of MAP (–12±3%), RBF (+23±7%), CLDF (+9±4%), and MLDF (–12±7% change) were indistinguishable from those of rabbits receiving candesartan alone (Figure 1).

Candesartan treatment increased ERBF (by 31±12% across both kidneys) and reduced filtration fraction (from 0.23±0.05 to 0.18±0.03 across both kidneys) but did not significantly affect GFR, urine flow, or sodium excretion. Neither vehicle nor PD123319 treatment altered these variables, and PD123319 treatment did not alter the profile of responses to candesartan (data not shown).

Responses to Infusion of Angiotensin Peptide Agonists
In vehicle-treated rabbits, none of the agonist infusions into the left renal artery significantly altered MAP, HR, ERBF, GFR, filtration fraction, urine flow, or sodium excretion in the noninfused (right) kidney. The one exception to this was

![Figure 1. Effects of antagonist treatments on systemic and renal hemodynamics. Data indicate percentage differences between levels during a 10-minute control period and those 20 to 30 minutes after antagonist treatments commenced. Columns and error bars represent mean±SEM (n=6). MAP indicates mean arterial pressure; RBF, total renal blood flow; CLDF, cortical laser Doppler flux; MLDF, medullary laser Doppler flux; and Cand, candesartan. **P<0.01, ***P<0.001 for change from control (paired t test).](image-url)
MLDF returned to its control level (52±9 U). However, responses of RBF and CLDF to angiotensin III were indistinguishable from those in vehicle-treated rabbits (Figure 3).

In vehicle-treated rabbits, angiotensin II (1–7) slightly reduced RBF (by 4±1% at 500 ng kg⁻¹ min⁻¹) and CLDF (by 7±2%) but did not significantly affect MLDF. These responses were abolished by candesartan. CGP42112A did not significantly affect any of the measured variables. Responses to angiotensin II (1–7) and CGP42212A were not significantly altered by PD123319 treatment (data not shown).

**Responses to Bolus Doses of Angiotensin Peptide Agonists**

No changes in MAP or HR were observed in response to these agents, but profound changes in RBF, CLDF, and MLDF were observed. In vehicle-treated rabbits, angiotensin II (50 ng/kg) caused MLDF to initially decrease during the 30 seconds after it was administered but then increase to levels greater than control during the following ~70 seconds (Figure 4). This was accompanied by decreases in RBF and CLDF (Figures 4 and 5). Similar responses were induced by angiotensin III (250 ng/kg; Figure 6), but neither angiotensin II (1–7) (1000 ng/kg) nor CGP 42112A (1000 ng/kg) had discernible effects on renal hemodynamic variables (data not shown). Responses to angiotensin II and angiotensin III were greatly blunted by candesartan treatment, whether it was given alone or in combination with PD123319. PD123319 did not significantly affect responses to the angiotensin peptides.

**Discussion**

The notion that AT₂-mediated vasodilatation blunts AT₁-mediated vasoconstriction is well established, particularly in the context of arterial pressure regulation. Within the kidney, AT₂ receptor activation can stimulate a variety of vasodilator signaling cascades, including the bradykinin/nitric oxide/cGMP cascade, phospholipase A₂, and production of epoxyeicosatrienoic acids. There is also evidence for a small reduction in sodium excretion during CGP42112A infusion (19±8% at 500 ng kg⁻¹ min⁻¹; data not shown). In the left (infused) kidney, ERBF fell (by 60±10% at 125 ng kg⁻¹ min⁻¹) and filtration fraction increased (from 0.34±0.05 to 0.39±0.05) during infusion of angiotensin III, but no other significant changes in clearance variables were observed with any of the peptides (data not shown). In contrast, profound effects of angiotensin II and angiotensin III on RBF, CLDF, and MLDF were observed.

In vehicle-treated rabbits, renal-arterial infusion of angiotensin II was accompanied by dose-dependent decreases in RBF (by 37±10% at 25 ng kg⁻¹ min⁻¹) and CLDF (by 39±10%) but not MLDF (−12±9% change). These responses were virtually abolished by candesartan treatment, whether given alone or combined with PD123319. When administered alone, PD123319 treatment significantly enhanced angiotensin II–induced reductions in RBF (69±3% at 25 ng kg⁻¹ min⁻¹) and tended to enhance reductions in CLDF (65±10%) but also uncovered a dose-dependent angiotensin II–induced rise in MLDF, which increased from 42±7 U (control) to 52±6, 57±8, and 61±7 U, respectively, during infusion of 1, 5, and 25 ng kg⁻¹ min⁻¹ angiotensin II. During the recovery period, MLDF returned to its control level (47±3 U; Figure 2).

Infusion of angiotensin III caused dose-dependent decreases in RBF (51±8% at 125 ng kg⁻¹ min⁻¹) and CLDF (50±11%) but not MLDF (−9±12% change). These effects were abolished by candesartan, whether it was given alone or combined with PD123319. In PD123319-treated rabbits, MLDF increased from 52±11 U (control) to 58±12, 69±15, and 76±16 U, respectively, during infusion of 5, 25, and 125 ng kg⁻¹ min⁻¹ angiotensin III. During the recovery period,
that AT₂ receptors can mediate vasodilatation in the renal vasculature in vitro. However, until now there has been little evidence for AT₂-mediated renal vasodilatation in vivo. In the present study, we found that PD123319 enhanced angiotensin II–induced reductions in RBF (and tended to enhance reductions in CLDF), which were themselves completely abolished by candesartan. Thus, AT₂-mediated vasodilatation appears to blunt AT₁-mediated vasoconstriction in the renal cortex.

We hypothesized that AT₂-mediated vasodilatation also contributes to the actions of angiotensin peptides in the medullary circulation. We now reject this hypothesis, because PD123319 did not unmask AT₂-mediated reductions in MLDF during renal-arterial infusion of angiotensin II or angiotensin III or blunt increases in MLDF after bolus doses of these peptides. Indeed, our data suggest that AT₂ receptors actually oppose AT₁-mediated vasodilatation in the medullary microcirculation, because PD123319 unmasked dose-dependent increases in MLDF in response to angiotensin II and angiotensin III, which were abolished by candesartan. Thus, AT₂ receptor activation appears to counteract both vasodilator and vasoconstrictor responses within the kidney mediated by AT₁ receptors in a regionally specific manner.

The medullary circulation is unique, in that it can respond to angiotensin II with profound vasodilatation. Angiotensin II–induced increases in MBF have been described previously in both rats and rabbits. Our present results indicate that this is also true in rabbits, because candesartan blunted the increases in MLDF observed after bolus renal-arterial administration of angiotensin II and angiotensin III and when these peptides were infused into the renal artery after PD123319 treatment. Furthermore, angiotensin II (1–7) and CGP42112A, which have relatively high affinity for AT₂ receptors, did not significantly affect MLDF. This latter finding is also crucial for interpreting our observation that PD123319 uncovered increases in MLDF during infusions of angiotensin II and angiotensin III. AT₂-mediated renal vasoconstriction has been reported under some experimental conditions, but our results indicate that AT₂ receptor activation does not have direct vasoconstrictor actions in the medullary circulation under the present experimental conditions. Rather, our data are consistent with the novel concept that AT₂ receptor activation can inhibit AT₁-mediated vasodilatation within the medullary vasculature.

There is strong evidence that angiotensin II–induced vasodilatation in the vascular elements that control MBF is mediated by release of nitric oxide and/or prostaglandins. Furthermore, AT₁ receptor activation can induce nitric oxide release from the vasculature in vitro. Our data regarding the effects of PD123319 therefore suggest that AT₂ receptor activation can blunt AT₁-mediated release of vasodilator factors that act on vascular elements controlling MBF. At face value, this conclusion seems at odds with recent in vitro findings by Rhinehart et al in isolated, outer medullary, descending vasa recta, vascular elements that are probably important in the control of MBF. They showed that AT₂ receptor activation blunted vasoconstrictor responses to angiotensin II in these vessels and also opposed angiotensin
II–induced suppression of intracellular Ca\(^{2+}\) concentration in endothelial cells. This is consistent with the notion that AT\(_2\) receptor activation mediates vasodilatation in outer medullary, descending vasa recta by promoting endothelial nitric oxide release. However, the nitric oxide that opposes angiotensin II–induced vasoconstriction in vascular elements controlling MBF is probably chiefly derived from the tubular epithelium rather than the vascular endothelium.\(^{39,40}\) Tubular elements are excluded from the isolated, descending vasa recta preparation. Our present observations, therefore, suggest that the AT\(_2\)-mediated vasodilator mechanism identified by Rhinehart et al in the medullary vasculature in vitro might be masked in the intact kidney by AT\(_2\)-mediated inhibition of tubulovascular nitric oxide cross-talk.

PD123319 had no detectable effect on resting systemic or renal hemodynamics, and although candesartan reduced resting MAP and increased RBF and CLDF, it had no detectable effect on MLDF. Thus, neither AT\(_1\) nor AT\(_2\) receptors appear to contribute greatly to the control of resting MBF in rabbits under these experimental conditions. This also appears to be the case in anesthetized rats.\(^{6}\)

Nevertheless, our results indicate that AT\(_2\) receptor activation can modulate renal vascular responses to AT\(_1\) receptor activation in a regionally specific manner. Thus, AT\(_2\) receptor activation appears to blunt both cortical vasoconstriction and vasodilatation in vascular elements controlling MBF, both of which are mediated by AT\(_1\) receptors. This type of cross-talk between AT\(_1\) and AT\(_2\) receptors differs from the conventional view of AT\(_2\) receptor function, because AT\(_2\)-mediated effects are often only detectable once AT\(_1\) receptors have been blocked,\(^{15,32,41}\) indicating that they are mediated independently of AT\(_1\) receptors. In contrast, in the rabbit renal vasculature in vivo, the effects of AT\(_2\) receptor activation could only be detected on a background of AT\(_1\) receptor activation, reflecting a modulatory role of AT\(_2\) receptors rather than a direct influence on renal vascular tone.

The response of the medullary microcirculation to exogenous angiotensin II appears to depend on its mode of delivery. For example, high-dose intravenous infusions of angiotensin II in rabbits transiently increase MLDF, which returns to control levels within 10 to 20 minutes despite continuous delivery of angiotensin II.\(^{3,42}\) When doses of angiotensin II are increased from a threshold level, MLDF remains remarkably stable in the face of profound reductions in RBF and CLDF.\(^{19}\) When given as a renal-arterial bolus, angiotensin II decreases total RBF and CLDF but has a biphasic effect on MLDF, with an initial decrease followed by an increase.\(^{10,11}\) Our present data indicate that angiotensin II–induced increases in MLDF are mediated by AT\(_1\) receptors, not AT\(_2\) receptors. Although PD123319 treatment uncovered increases in MLDF during renal-arterial infusions of angiotensin II and angiotensin III, it did not significantly affect responses to renal-arterial boluses of these peptides. One possible explanation for this discrepancy is that responses to these relatively high doses of angiotensin II and angiotensin III were already maximal. However, our data would also be consistent with a role for AT\(_2\) receptors in mediating the rapid inhibition of AT\(_1\)-mediated vasodilatation, either through a direct interaction with AT\(_1\) receptors or with their signaling cascades. Taken together, these observations suggest that the AT\(_1\) receptor mechanism that mediates medullary vasodilatation in rabbits is normally rapidly downregulated during prolonged exposure to angiotensin II. This does not appear to be the case in rats, in which intravenous infusions of angiotensin II produce sustained increases in MBF, which are little affected by PD123319.\(^{6–8}\)

We must also consider the limitations of our experimental approach. In addition to its endocrine role, angiotensin II has important paracrine/autocrine roles in the kidney. Indeed, interstitial and tubular concentrations of angiotensin II far exceed those in plasma.\(^{43}\) Effects of renal-arterial infusions of angiotensin peptides might therefore not always reflect those of activation of the intrarenal renin-angiotensin system. The renal-arterial infusion method also has the potential for plasma streaming to confound the effects of vasoactive agents.\(^{44}\) However, it is unlikely that this confounded our present observations, because the effects of renal-arterial infusion of angiotensin II on regional kidney blood flow closely resembled those observed during intravenous infusions,\(^{3,10,42}\) and we systematically tested multiple agents under identical conditions. We must also consider the limitations of laser-Doppler flowmetry, which in the kidney chiefly reflects erythrocyte velocity rather than bulk blood flow per se.\(^{45}\) Thus, MLDF would not provide a quantitative measure of changes in bulk blood flow if the number of perfused vasa recta or dynamic hematocrit changed. We can probably exclude the latter possibility and that our observations were confounded by changes in water reabsorption within the medulla (which could in turn alter blood flow), because none of the treatments that we administered altered GFR or urine flow.

**Perspectives**

Our results indicate that, under resting conditions in normotensive rabbits, endogenous angiotensin peptides have little influence on MBF. In contrast, our data indicate strikingly different roles for AT\(_1\) and AT\(_2\) receptors in mediating responses to exogenous angiotensin peptides in the cortical and medullary circulations of the rabbit kidney. AT\(_1\) receptor activation appears to counteract both AT\(_2\)-mediated vasoconstriction in the cortex and AT\(_2\)-mediated vasodilatation in the medulla. The influence of AT\(_1\) and AT\(_2\) receptor activation on MBF under conditions of increased renin-angiotensin system activity (eg, salt depletion or renovascular hypertension) remains unknown. This issue requires resolution, particularly given that AT\(_2\) receptor activation has an antihypertensive effect in Grollman hypertension\(^{14}\) and can enhance the antihypertensive efficacy of AT\(_1\) blockade\(^{15,41}\) and that responses of MBF to angiotensin II are altered in hypertension.\(^{9}\)

**Acknowledgments**

This work was supported by grants from the National Health and Medical Research Council of Australia (143785, 143603, 143564) and the Ramaciotti Foundations (A6370, RA159/98, RA032/01). Lisa Duke is a Monash Graduate Scholar.

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Hypertension. 2003;42:200-205; originally published online July 7, 2003;
doi: 10.1161/01.HYP.0000083341.64034.00
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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