Renal Vascular Reactivity in Mice: AngII-Induced Vasoconstriction in AT1A Receptor Null Mice

XIAOPING RUAN,* MICHAEL I. OLIVERIO,† THOMAS M. COFFMAN,† and WILLIAM J. ARENDSHORST*  
*Department of Cell and Molecular Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; and †Department of Medicine, Duke University, Durham, North Carolina.

Abstract. The present study describes methodology and its application to evaluate renal reactivity in acute studies on anesthetized mice. Renal blood flow (RBF) was measured using an ultrasonic transit-time flowmeter and a non-cannulating V-shaped probe. An intrarenal artery injection technique established feasibility and reproducibility of studies of renal vascular reactivity to angiotensin II (AngII) in adult wild-type mice. The study also examined whether AngII would affect RBF in mice lacking AT1A receptors due to gene targeting. Mean arterial pressure averaged 83 and 62 mmHg, respectively, in mice with and without AT1A receptors. The RBF was similar in both groups, averaging 7 ml/min per g kidney wt. AngII injection (10-μl bolus) into the renal artery produced transient, dose-dependent, selective reductions in RBF in AT1A knockout mice as well as wild-type mice. The response was considerably greater in mice with AT1A receptors: 10% for 0.1 ng, 30% for 1 ng, and 45% for 5 ng AngII in control animals versus respective decreases of 6, 15, and 17% in knockout mice. In other studies, angiotensin-converting enzyme (captopril) or renin (CP-71362-14) was inhibited. During inhibition of AngII formation, renal vascular reactivity to AngII increased twofold in both groups. Coadministration of the AT1 receptor antagonist losartan (1 to 1000 ng) elicited dose-dependent inhibition of AngII effects, with near maximum blockage of 80 to 90% in both groups of mice. The putative AT2 receptor antagonist PD 123319 inhibited 30 to 40% of AngII-induced vasoconstriction, whereas CGP 42112 had no effect in either group. In conclusion, AngII can elicit renal vasoconstriction, albeit attenuated, in AT1A knockout mice. The weaker RBF effects are most likely due to the absence of the AT1A receptor. Inhibition of the response by AT1 receptor antagonist suggests mediation by the AT1B receptor in these animals. The residual constrictor effect observed during AT1 receptor blockade and sensitive to PD 123319 appears to be mediated by a non-AT1 receptor.

In the past decade, molecular biology techniques have led to an explosion of attractive animal models for research. The challenge for the next decade centers on assessment of integrated organ function such that the phenotypes of transgenic animals with genetically engineered loss or gain of function can be characterized in a comprehensive manner. From a genetic perspective, the mouse is the animal of choice for gene targeting. On the other hand, the mouse poses challenging technical hurdles that have tended to impede in-depth studies of systems physiology, such as cardiovascular and renal function. A high level of skill and careful attention to detail are required because of a mouse’s small size—a 30 g body wt of an adult with each kidney weighing less than 0.3 g, with renal arteries between 0.3 to 0.5 mm in diameter and a blood volume of 2.0 to 2.5 ml. Recent reports indicate that systemic arterial pressure is influenced by interruption of enzyme systems, such as those involved in the production of angiotensin II (AngII), nitric oxide, or endothelin, and also by deletion of receptors such as AngII, bradykinin, endothelin, and atrial natriuretic peptide (1–7).

Hemodynamic studies on specific regional vascular beds in mice have been limited in part by the availability of reliable miniaturized instrumentation and measurement devices. A major advance includes refinement of small diameter, non-cannulating flow transducers capable of accurate and reproducible recordings of pulsatile and mean renal blood flow (RBF) in anesthetized mice (8). Such methodology for the mouse should complement earlier hemodynamic studies on larger rodents. These investigations have characterized rapid, transient auto-regulatory responses of the renal vasculature to acute changes in arterial pressure, frequency response times of intrarenal controllers of vascular tone, and vascular reactivity assessed as transient and steady-state responses to systemic or intrarenal administration of vasoactive agonists and antagonists.

It is well known that the octapeptide AngII is an important effector in renin-angiotensin-aldosterone cascade, exerting its biologic actions on target cells after binding to and activating cell surface receptors. Which specific receptors mediate actions of AngII on various cell types is an area of considerable interest and active investigation. The extent to which the relative distribution of AT1 receptor subtypes is associated with similar or different signal transduction pathways and ultimately

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Correspondence to Dr. William J. Arendshorst, Department of Cell and Molecular Physiology, CB #7545, School of Medicine, Room 152, Medical Sciences Research Building, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7545. Phone: 919-966-1067; Fax: 919-966-4960; E-mail: arends@med.unc.edu

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with functions in mammalian cells is not known. Although pharmacologic agents are reported to be selective for \( \text{AT}_1 \) and \( \text{AT}_2 \) receptor types, currently available \( \text{AT}_1 \) receptor antagonists do not distinguish between \( \text{AT}_{1A} \) and \( \text{AT}_{1B} \) subtypes. Gene targeting is a powerful alternative approach allowing exploration of the functional significance of distinct AT types/subtypes \textit{in vivo}. To this end, mouse models have been developed that exhibit homozygous and heterozygous mutations of the \( \text{AT}_{1A} \) receptor gene to render it nonfunctional (9–12). As predicted, the absence of \( \text{AT}_{1A} \) receptors leads to hypotension and elevated plasma renin activity. A distinct functional role of the \( \text{AT}_{1B} \) receptor is suggested by the relatively normal renal morphology and mild pelvic enlargement compared with mice lacking angiotensinogen or both \( \text{AT}_1 \) receptor subtypes (7,13). Mice with their \( \text{AT}_2 \) receptors deleted by gene targeting present with normal morphology and slight hypertension (6,14).

In this regard, the \( \text{AT}_{1A} \) receptor null mouse model provides a unique opportunity to better understand AngII effects and mediation by \( \text{AT}_{1B} \) receptors and possibly other receptors in the absence of a functional \( \text{AT}_{1A} \) Receptor (9,12). It is not clear whether the \( \text{AT}_{1B} \) receptor mediates any of the effects of AngII on cardiovascular and renal function. It is reported that \( \text{AT}_{1B} \) knockout animals have a normal arterial pressure (5). On the other hand, \( \text{AT}_1 \) receptor antagonists inhibit AngII-stimulated cytosolic calcium concentration in cultured aortic smooth muscle cells derived from \( \text{AT}_{1A} \) knockout mice (15). \textit{In vitro} studies suggest that there may be no difference in the functional properties of \( \text{AT}_1 \) receptor subtypes, at least those separately transfected into Chinese hamster ovary cells or adrenocortical Y-1 cells (16,17). The transfected \( \text{AT}_{1B} \) receptor subtype is known to activate the phosphoinositide-calcium second-messenger signaling pathways as does the \( \text{AT}_{1A} \) receptor. However, these cells are unique and may differ markedly from receptor function and signaling pathways in natural vascular smooth muscle cells of a resistance arteriole.

The purpose of the present report was twofold. One goal was to establish feasibility of a method to provide continuous measurement of RBF in conjunction with an intrarenal injection technique previously used in rats. Specifically, we wanted to measure renal hemodynamics using a small non-cannulating flow transducer interfaced with a transit-time flowmeter system to assess renal vascular reactivity to AngII \textit{in vivo} in anesthetized mice. A second objective was to evaluate whether AngII would elicit renal vasoconstriction in mice lacking functional \( \text{AT}_{1A} \) receptors and, if so, to compare the magnitude of the responses to wild-type mice. Moreover, we used pharmacologic antagonists to characterize the functional role of distinct AngII receptors or subtypes in the regulation renal vascular motor tone in both wild-type control mice and homozygous \( \text{AT}_{1A} \) knockout mice with \( \text{AT}_{1A} \) receptors deleted by gene targeting.

**Materials and Methods**

Experiments were performed on anesthetized adult mice with or without \( \text{AT}_{1A} \) receptor gene mutated by gene targeting techniques. \( \text{AT}_{1A} \)-deficient mice were generated by homologous recombination and compared with genetically matched littermates of randomly mixed background (C57Bl6/129) of the F2 generation as originally described by Ito et al. (9). The animals were given tap water \textit{ad libitum} but were deprived of food overnight before an experiment. All animal protocols are in accordance with the University of North Carolina at Chapel Hill Institutional Guidelines (IACUC Approval 96-07-0 and 99-030-0).

The experimental preparation was as described previously (18). In brief, anesthesia was induced by an intraperitoneal injection of sodium pentobarbital (65 mg/kg body wt) diluted 5 to 10 times, and the animals were placed on a servo-controlled heating table that maintained body temperature at 37°C. A tracheostomy was performed to facilitate free breathing. The right carotid artery was cannulated to obtain blood samples and monitor arterial pressure (Statham P23Db transducer, Costa Mesa, CA). The right jugular vein was cannulated for the administration of maintenance infusions and supplemental doses of sodium pentobarbital. Isotonic bovine serum albumin (4.7 g/dl) was infused, initially at a rate of 10 μl/min to replace losses associated with surgery, and then at 2 μl/min for the duration of an experiment to maintain hematocrit and plasma protein concentration at presurgical levels. Midline and subcostal incisions were used to expose the abdominal aorta and left kidney. The left kidney was covered with saline-soaked cotton, and the renal artery was carefully separated from the renal vein. A tapered and curved PE 10 catheter was introduced into the aorta until its tip was positioned approximately 0.5 mm into the left renal artery. Blood flow measurements verified that catheter placement in the renal artery did not affect renal hemodynamics. The renal arterial catheter was used for the local administration of test agents. Proper catheter placement was verified at the start of the control period. Throughout the experiment, a continuous infusion (1 μl/ min) of heparinized isotonic saline was administered via the renal arterial catheter. A Cheminert sample injection valve (Valco Instruments Co., Houston, TX) was used to introduce a 10-μl bolus into the infusion line. After completion of the surgical preparation, the animals were allowed to stabilize for 30 min before starting the measurements.

Total blood flow in the left renal artery was measured using a V-shaped (size 0.5 V) non-cannulating flow probe connected to an ultrasonic transit-time flowmeter (Transonic Systems, Inc., Ithaca, NY) (8,18). An optimal acoustical coupling between the renal artery and the bottom notch in the V-shaped probe was ensured by a viscous jelly. Each blood flow recording was started at the time AngII was introduced into the renal artery infusion line. One minute before the administration of AngII alone or together with \( \text{AT}_1 \) or \( \text{AT}_{2} \) antagonists, the rate of saline infusion was increased from 1 to 30 μl/min so that the entire bolus (10 μl) of AngII reached the kidney by 30 s. After recovery of RBF to its baseline level, usually by 3 min, the renal arterial infusion rate was returned to 1 μl/min. In acceptable injections and recordings, the vascular actions were localized to the kidney and had no effect on systemic arterial pressure. The flow signal was amplified (Medium Gain Hewlett Packard 8802A amplifier, Avondale, PA) and recorded on a Hewlett Packard 7414A 4-channel oscillograph and interfaced with a Pentium IBM-compatible computer and data acquisition AD board. The data were subsequently processed by Labtech Notebook Software (Andover, MA) (18–20).

Various doses of the AngII receptor antagonists were injected as a mixture with 100 pg to 10 ng of AngII. The order of doses was randomly selected each day. The time interval between injections was 10 min. Preliminary studies established that intrarenal injections of all doses of the AngII receptor antagonists given alone did not affect baseline arterial pressure or RBF. To avoid treatment interactions, only one AngII receptor antagonist was used in a given mouse. To
evaluate whether consecutive injections of an antagonist created an additive buffering effect, responses to AngII alone were evaluated at the beginning, middle, and end of an experiment. In all cases, AngII produced similar decreases in renal blood flow over time.

The baseline (pre-injection) values for blood flow, arterial pressure, and vascular resistance were calculated separately for each injection. Control values were averaged during the time period between the introduction of AngII into the infusion line and the onset of renal vasoconstriction. Subsequent changes in flow, pressure, and resistance were normalized and expressed as a percentage of baseline values. Responses were plotted in the time domain using the SigmaPlot software package.

The following drugs were used: AngII and captopril (Sigma Chemical Co., St. Louis, MO), losartan (DuPont-Merck Pharmaceutical Co., Wilmington, DE), PD 123319 (Parke-Davis, Ann Arbor, MI; Research Biochemical International, Natick, MA), CGP 42112 (Ciba Geigy, Basel, Switzerland), and CP-71362–14 (Pfizer, Groton, CT). Dose–response relations were obtained for AngII and angiotensin receptor antagonists. Doses of AngII receptor antagonists, captopril, and CP-71362–14 were based on previous studies on rodents (19,21–23).

**Statistical Analyses**

Statistical analyses were performed using a SigmaStat software package (SPSS, Chicago, IL). When evaluating multiple factors (e.g., mouse strain and drug concentration) between two groups, data analyses were performed by two-way ANOVA. When evaluating AngII or AngII receptor antagonist-related effects within groups, data were analyzed by one-way ANOVA and ANOVA for repeated measures, with post hoc testing via Student-Newman-Keuls. Paired and unpaired single treatment effects within animals and between groups were analyzed by paired and unpaired t tests, respectively.

**Results**

Renal vascular reactivity to a standard dose of AngII was determined over time in the absence and presence of pharmacologic antagonists of the renin-angiotensin system. A typical response to AngII (1 ng) administered as a 10-μl bolus into the renal artery is shown in Figure 1. AngII reaches the kidney after roughly a 30-s delay. Maximum constrictor responses of about 30 to 40% are noted approximately 60 s after the injection. By design, the experimental conditions were such that this percent decrease in RBF is similar to that elicited by 1 to 2 ng of AngII in the rat. Thereafter, renal vascular resistance usually recovered completely by 3 min. Intrarenal injection of AngII produced local changes in RBF and vascular resistance without affecting systemic arterial pressure.

Other studies were performed to characterize the dose–response relation of Ang II and RBF in the mouse. Figure 2 presents renal hemodynamic responses to three doses of AngII injected randomly into the renal artery of an anesthetized wild-type mouse. The 0.1-ng dose produced a 10% reduction in flow, and the 5-ng dose decreased RBF by 50%. Most of our subsequent experiments used the intermediate dose of 1 ng that usually produced a 30% decrease in RBF.

Additional experiments evaluated whether AngII-induced renal vasoconstriction was reproducible over time in a given experiment. Three injections were made using two different doses of AngII; subsequent injections were separated by at least 10 min. Figure 3 shows that the responses to each dose were highly reproducible during the course of an experiment. AngII (1 ng) reduced RBF by 30 to 35% baseline flow; the

![Figure 1.](image-url) Representative tracing indicating that angiotensin II (AngII) produces a local change in renal blood flow (RBF) and vascular resistance independent of a change in mean systemic arterial pressure in a wild-type mouse.
5-ng bolus reduced blood flow by 60 to 65%. These observations validate the injection technique and verify the stability of the preparation over a 2- to 3-h time period postsurgery.

A second goal of our study was to characterize renal vascular reactivity to AngII mediated by the AT1 receptors in wild-type mice and to determine whether AngII exerts an effect in mice lacking the AT1A receptor due to gene targeting. Table 1 presents baseline hemodynamic data for anesthetized mice averaging 30 g body wt. Mean arterial pressure was lower in AT1A knockout mice (62 versus 83 mmHg, P < 0.001). However, there was no major difference in RBF between genotypes, averaging 1.5 to 1.6 ml/min per kidney or 6 to 8 ml/min per g kidney wt. Renal vascular resistance was significantly lower in the AT1A null mice, paralleling arterial pressure. Hematocrit did not differ between groups.

The data are grouped according to gender in Table 2 and Figure 4. Within genotypes, mean arterial pressure did not differ, although RBF, body weight, left kidney weight, and hematocrit tended to be slightly lower in females. When normalized to kidney mass, there were no significant gender differences in RBF or renal vascular resistance within AT1A (+/+), wild-type and AT1A (−/−) mutant groups (Figure 4, Table 2).

To determine whether AngII would affect RBF in the absence of the AT1A receptor, renal vascular reactivity was assessed by intrarenal injection of different amounts of AngII. As shown in Figure 5, intrarenal injection of 0.1 and 1 ng of AngII reduced RBF in a dose-dependent manner by 10 and 30% in AT1A (+/+) control mice. In marked contrast, AngII caused weak renal vasoconstriction in mice lacking the AT1A receptor, with 6 to 15% reductions in blood flow. A higher dose (5 ng) had no additional effect in AT1A-deficient mice (17% reduction in RBF), whereas AngII-induced renal vasoconstriction increased to 45% in wild-type mice. Thus, the magnitude of the RBF changes in the knockout mice was markedly attenuated compared with renal hemodynamic responses in wild-type mice, and attained maximum effects were considerably attenuated in the mutant animals.

To examine whether the attenuated effect of exogenous AngII is due to the influence of endogenous AngII in AT1A knockout mice, paired RBF experiments were conducted in mice before and after inhibition of endogenous AngII production. Figure 6 (left panel) illustrates that intravenous injection of captopril (1 mg/kg) to inhibit angiotensin-converting enzyme (ACE) magnified the renal vascular responses to AngII; the effect of ACE inhibition was similar in both groups of mice. In the control period, AngII (1 ng) reduced RBF by 21 ± 6% in wild-type mice. After 30 min of ACE inhibition with captopril, the same dose of AngII caused a twofold greater reduction in RBF (41 ± 6%, P < 0.01). Similarly, in AT1A receptor-deficient mice, the response to AngII (1 ng) was increased twofold, from 7 ± 2% to 14 ± 2% (P < 0.005) during ACE inhibition.

In other studies, the effect of AngII (1 ng) was evaluated before and after renin inhibition. In these studies, CP-71362 was infused into the renal artery for 30 min at 5 μg/kg per min to inhibit production of endogenous AngII. As shown in Figure 6 (right panel), the renin inhibitor doubled AngII-induced renal vasoconstriction from 25 ± 1% to 40 ± 3% in control mice (P < 0.02) and from 9 ± 3% to 18 ± 5% in knockout mice.
The effect of the renin inhibitor was similar to that reported above for ACE inhibition. ACE or renin blockade led to equivalent increases in vascular reactivity to administered AngII in the two groups of mice.

Additional experiments determined which particular receptor type or subtype is responsible for AngII-induced renal vasoconstriction in mice lacking the AT1A receptor. To this end, AngII was given alone or coadministered with a selective AT1 or AT2 receptor antagonist. The inhibitory effect of the AT1 receptor antagonist losartan is summarized in Figure 7.

During the control period, AngII (1 ng) decreased RBF by 27 ± 8% in control mice. A higher dose of AngII (10 ng) was required to reduce renal perfusion to a similar amount (22 ± 9%) in AT1A knockout mice. These doses elicited similar reductions in RBF reduction, which were defined as 100% AngII effect for these studies. Losartan (1 to 1000 ng) blocked AngII-induced renal vasoconstriction in a dose-dependent manner. The magnitude of the inhibition was similar in control and knockout mice: Maximum inhibition approached 80 to 85%. This finding is consistent with our previous observations with candesartan, another AT1 receptor blocker (18).

The two putative AT2 receptor antagonists exhibited different effects. PD 123319 (100 to 1000 ng) blocked a significant portion of AngII-induced renal vasoconstriction. The inhibition was dose-dependent, attenuating the AngII effect up to 35 ± 5% in control mice and 43 ± 8% in AT1A knockout mice (Figure 8). These values do not differ statistically between strains (P > 0.1). In contrast, another AT2 receptor ligand, CGP 42112 (1000 ng), had no significant effect on AngII-induced renal vasoconstriction in either group when given as a bolus simultaneously with AngII (P > 0.2). Administration of CGP 42112 alone had no effect on RBF when given in doses up to 1000 ng. These results for the mouse are in close agreement with our previous findings in rats (19,24).

Discussion

The current study used advanced technology in flowmetry to measure absolute as well as relative changes in blood flow through vessels as small as the mouse renal artery. The size of the non-cannulating flow transducer allows its application to vessels smaller than 0.5 mm in diameter. As noted below, previous studies have characterized RBF in the mouse under steady-state conditions (18,25–27). What is unique in our study is the combination of continuous RBF measurement along with injection of a vasoactive agent such as AngII directly into the renal circulation. This paradigm allows quantitative assessment of localized dynamic responses of renal vasomotor tone to relatively small amounts of test substance independent of systemic mechanisms that may act to buffer changes in arterial pressure and possibly elicit secondary renal changes, thereby complicating data interpretation. Also, immediate direct effects are recorded and compared to steady-state responses that often include secondary effects representing compensation by other vasoactive systems.

In our study, mean arterial BP of anesthetized adult wild-type mice was 83 mmHg with a RBF of 6 to 8 ml/min per g kidney wt. This range of steady-state RBF is similar to results reported by other investigators using the ultrasonic system or various other techniques including clearance and microsphere (1,18,26,27). In addition to these techniques, other groups have used fluorescence microscopy to investigate the renal cortical circulation in mice and renal vascular reactivity in isolated perfused mouse kidneys (28,29). Several recent, more detailed mouse studies have investigated steady-state adjustments in RBF to chronic changes in salt diet and to acute changes in arterial pressure as well as to vasoactive agents such as bradykinin and endothelin using ultrasonic transit-time and laser Doppler flowmetry in normal mice (1,26–28). The ultrasonic flowmeter system also has been used to determine the steady-state hemodynamic effects of bradykinin in mice with B2 receptors mutated by gene targeting (1). AT1A knockout mice have a lower mean arterial pressure compared with controls, in agreement with published values (9,10). Interestingly, there is no major difference in RBF between mice with or without AT1A receptors. These basal hemodynamic data also suggest that RBF autoregulation in the mouse is efficient in the absence of an increased cardiac output.
of AT\textsubscript{1A} receptors and that there is no absolute requirement for AT\textsubscript{1A} receptors in such intrinsic regulation. In contrast, AT\textsubscript{1A} receptors seem to be necessary for the operation of tubuloglomerular feedback and its control of preglomerular vascular resistance (30). In addition, kidney development including the vasculature in AT\textsubscript{1A} knockout mice is basically normal, except for slight papillary hypoplasia and hyperplasia of renin-producing granular cells. The morphologic abnormalities are relatively mild in nature compared to the major changes observed in mice deficient in angiotensinogen or lacking both AT\textsubscript{1A} and AT\textsubscript{1B} receptors (7,13).

We assessed effects of AngII on the renal vasculature in wild-type and AT\textsubscript{1A} null homozygotes. Responses in control mice in terms of percent change in RBF are similar to those we have observed previously in the anesthetized rat (18–20,24,31). In our previous studies, reverse transcription-PCR data showed that the ratio of AT\textsubscript{1A}/AT\textsubscript{1B} is 4:1 in freshly isolated preglomerular arterioles of rats (19) and 2:1 in cultured aortic smooth muscle cells from wild-type mice (15). RNase protection assays indicated a ratio of 1.0 for glomeruli and 0.7 for the cortex of rat kidneys (32). Whole kidney tissue of mice expresses much more AT\textsubscript{1A} mRNA than AT\textsubscript{1B} message (33). To our knowledge, the relative abundance of AT\textsubscript{1A} to AT\textsubscript{1B} receptors is not known for the renal cortex or renal resistance vessels in the mouse. We provide new information about the contribution of different AngII receptors to AngII-induced renal vasoconstriction in both wild-type mice and mice lacking AT\textsubscript{1A} receptor.

Due to relative abundance, AT\textsubscript{1A} receptors are generally thought to mediate most AngII effects in almost all tissues and organs. However, currently available pharmacologic agents do not discriminate between AT\textsubscript{1} receptor subtypes, so it is not known whether AT\textsubscript{1A}, AT\textsubscript{1B}, or both are involved in the regulation of blood flow through various systemic capillary beds. Our results document that renal vascular reactivity to AngII is markedly attenuated in the absence of AT\textsubscript{1A} receptors, suggesting a predominant role for the AT\textsubscript{1A} receptor subtype in the mouse renal vasculature. On the other hand, we found that AngII is still able to produce some renal vasoconstriction in AT\textsubscript{1A} knockout mice. This observation implicates mediation

### Table 1. Ultrasonic flowmetry determination of renal hemodynamics in anesthetized mice\textsuperscript{a}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-Type AT\textsubscript{1A} +/+</th>
<th>Mutant AT\textsubscript{1A} −/−</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>83 ± 3</td>
<td>62 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RBF (ml/min per kidney)</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>RBF (ml/min per g kidney wt)</td>
<td>6.3 ± 0.4</td>
<td>7.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>RBF (ml/min per 100 g body wt)</td>
<td>5.4 ± 0.3</td>
<td>5.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>RVR (mmHg/(ml/min per g kidney wt))</td>
<td>13.1 ± 0.8</td>
<td>8.1 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>29 ± 1</td>
<td>30 ± 2</td>
<td></td>
</tr>
<tr>
<td>Left kidney weight (g)</td>
<td>0.23 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Hematocrit (ml/dl)</td>
<td>39 ± 1</td>
<td>39 ± 1</td>
<td></td>
</tr>
<tr>
<td>No. of mice</td>
<td>16</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance.

### Table 2. Renal hemodynamics in male and female mice\textsuperscript{a}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-Type AT\textsubscript{1A} +/+</th>
<th>Mutant AT\textsubscript{1A} −/−</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
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<tr>
<td>RBF (ml/min per kidney)</td>
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<td>RBF (ml/min per g kidney wt)</td>
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<tr>
<td>RVR (mmHg/(ml/min per g kidney wt))</td>
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<tr>
<td>Body weight (g)</td>
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<tr>
<td>Left kidney weight (g)</td>
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<tr>
<td>Hematocrit (ml/dl)</td>
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<tr>
<td>No. of mice</td>
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\textsuperscript{a}Abbreviations as in Table 1. P values for a gender difference are given in parentheses.
by the AT\textsubscript{1B} subtype because most of the AngII effect was inhibited by an AT\textsubscript{1} antagonist. The AT\textsubscript{1B} receptor appears to assume the primary role when AT\textsubscript{1A} receptors are absent. Consistent with our previous findings in normal rats (18,19,24), the AT\textsubscript{1} receptor antagonist losartan blocked up to 80 to 90\% of AngII-induced renal vasoconstriction in both control and AT\textsubscript{1A} knockout mice.

The attenuated hemodynamic effects of AngII in the mutant mice may be due in part to the elevated renin and endogenous AngII (9,11). The ability of AngII to produce vasoconstriction in these null mice becomes more readily apparent when high doses of AngII are given, especially during inhibition of ACE or renin activity. Two observations indicate that a mechanism(s) other than endogenous AngII concentration is responsible for the blunted effect of administered AngII in mice lacking AT\textsubscript{1A} receptors. First, AngII elicited less renal vasoconstriction in mutant mice both during control conditions and during ACE or renin inhibition. Second, AngII inhibition had equal augmenting effects in the two groups of animals. The combination of blunted renal reactivity and the apparent plateau of maximum effects with high AngII doses imply receptor-ligand saturation. Another explanation for the weak AngII effect relates to a reduced total AngII receptor density in the renal vasculature of AT\textsubscript{1A} mutant mice. Moreover, as discussed below, a heretofore undefined AngII receptor distinct from AT\textsubscript{1} and AT\textsubscript{2} receptors may mediate part of the response to AngII in rodents.

AT\textsubscript{1} and AT\textsubscript{2} receptors have been shown to exert opposite effects on cellular growth and differentiation, hormone release, neuronal activity, electrolyte transport, and vascular tone. Cardiovascular AT\textsubscript{2} receptors mediate vasodilation such that (1) AngII causes a depressor response during AT\textsubscript{1} receptor blockade, and (2) receptor antagonism with PD 123319 causes more or less vasoconstriction (34–38). Consistent with this view, AngII produces systemic vasodilation in AT\textsubscript{1A}/AT\textsubscript{1B} null mice (7), although another study reports no depressor effect (13), and constriction is seen in AT\textsubscript{2} knockout mice (6,14). Discrimination of receptor subtypes in these studies is based on selective inhibition of AngII binding by losartan but not PD 123319 or CGP 42112 in cells with only AT\textsubscript{1} receptors. Conversely, AngII binding to transfected AT\textsubscript{2} receptors is selectively inhibited by PD 123319 and CGP 42112 but not losartan.

The presence and functional role of AT\textsubscript{2} receptors in the renal circulation of adult animals is uncertain; results to date have been mixed. Some autoradiographic studies have localized a relatively small population of AT\textsubscript{2} receptors in large preglomerular vessels and even fewer in resistance arterioles (39). Low abundance of AT\textsubscript{2} mRNA in interlobular and afferent arterioles is indicated by reverse transcription-PCR (19). Functional studies give the impression that AT\textsubscript{2} receptors antagonized by PD 123319 buffer the constrictor action of AngII on isolated rat and rabbit afferent and efferent arterioles (37,40). However, earlier dog studies failed to uncover AT\textsubscript{2}-mediated effects on renal hemodynamics even when AngII levels were elevated by a low salt diet (41–43). Recent studies reveal an effect of AT\textsubscript{2} receptors on tubular transport (44–47).

At variance with the generally accepted view of vasodilatory AT\textsubscript{2} receptors, defined by PD 123319 antagonism, we and others have demonstrated that AngII can stimulate a PD 123319-sensitive receptor to elicit constriction of the vasculature of normal kidneys in adult rats. The present study extends this observation in mice with and without AT\textsubscript{1A} receptors. Other investigators have reported similar findings for renal resistance vessels of rats in vivo (19,24,48), the interlobular artery of the hydronephrotic rat kidney (49), and in isolated mesenteric arteries (50). Our previous rat studies demonstrated that the commonly used purported selective AT\textsubscript{2} receptor antagonist PD 123319 blocks about 20\% of AngII binding and AngII-induced renal vasoconstriction. We also found that the effects of an AT\textsubscript{1} receptor antagonist plus PD 123319 were additive, producing complete blockade of both AngII-induced renal vasoconstriction in vivo and radioligand binding in vitro (24). The current study extends these findings to the kidneys of mice, with similar data for wild-type mice and those deficient in AT\textsubscript{1A} receptors. Intermediate concentrations of the putative AT\textsubscript{2} receptor antagonist PD 123319 block about 30 to 40\% of the AngII effect in mice. To our knowledge, there is no evidence indicating a PD 123319 effect on AT\textsubscript{1} receptors unless pharmacologic amounts are tested (51–54). In contrast, the AT\textsubscript{2} receptor ligand CGP 42112 has no effect on AngII.

Figure 4. Comparison of group averages of mean arterial pressure (top panel) and renal blood flow (bottom panel) between male and female AT\textsubscript{1A} knockout and wild-type mice. Results are given as mean \pm SEM for more than seven mice per group. *\( P < 0.001 \) between groups.
binding in rat renal vessels, basal RBF, or AngII-induced renal vasoconstriction in mice or rats. It seems unlikely that all tested concentrations of this AT2 receptor ligand would always exert equally opposite antagonistic and agonistic effects. Thus, we conclude that the renal microcirculation has no AT2 receptors responsive to GCP 42112. To the extent that AT2 receptors are more prone to elicit vasodilation than contraction, the ability of PD 123319 to antagonize rather than potentiate AngII-induced renal vasoconstriction suggests mediation of a novel receptor. We propose that the rodent renal microcirculation has a unique PD 123319-sensitive receptor, distinct from conventional pharmacologically defined AT1 and AT2 receptors (19).

In terms of intracellular signaling, AT1 receptors on vascular smooth muscle cells are coupled to a Gq-protein that activates phospholipase C and liberates inositol trisphosphate and diacylglycerol. AT2 receptor signaling is less well understood and

Figure 5. Group averages of maximum reduction in RBF elicited by injection of three boluses of AngII (0.1, 1, and 5 ng) into the renal artery in adult anesthetized AT1A knockout (right panel) and wild-type (left panel) mice. Results are given as means ± SEM for more than five animals in each group.

Figure 6. Summary of the maximum changes of AngII-induced renal vasoconstriction before and after either captopril (angiotensin-converting enzyme inhibitor, 1 mg/kg, intravenously) or CP 71362 (renin inhibitor, 5 μg/kg per min, intrarenal infusion) treatment. Results are given as means ± SEM for four experiments in each group. *P < 0.01 inhibition versus control.
may differ among cell types. AT2 receptors, which appear to be upregulated in adults during volume contraction, are thought to be coupled to a Gi protein that is linked to production of kinins and nitric oxide and activation of guanylate cyclase to stimulate cGMP in the kidney (44,45) and in the coronary circulation (55–57). Other studies indicate that AT2 receptors stimulate arachidonic acid release and Na-K exchange in cultured proximal tubular cells (46,58), cells in which PD 123319 and CGP 42112 were equally effective in blocking AngII effects; both were more potent than losartan. In cardiomyocytes, PD 123319 blocks about 40% of inositol trisphosphate production stimulated by AngII, compared to 70% blockade by equimolar doses of losartan (59). In isolated rat renal microvessels, AngII stimulates production of the vasoconstrictor 20-hydroxyeicosatetraenoic acid, an effect inhibited by PD 123319 but not losartan (60). AngII-stimulation of cytosolic calcium is inhibited with equal effectiveness by PD 123319 and losartan in cultured rat mesangial cells (61).

In conclusion, we used an intrarenal injection technique combined with ultrasonic flowmetry to investigate renal reactivity to vasoactive agents in anesthetized mice. Bolus injection of small amounts of AngII into the renal artery of mice produce transient localized effects on renal hemodynamics that are reproducible, reversible, and of similar magnitude throughout an experiment. AT1A knockout mice exhibit a normal mean RBF in association with a lower mean arterial pressure under basal conditions, suggesting autoregulation of the renal vasculature independent of AT1A receptors. A new finding is that AngII is capable of eliciting renal vasoconstriction in AT1A null mice. The degree of vasoconstriction is markedly attenuated, and the maximum effect plateaus at approximately a 15% reduction in RBF, compared with complete ischemia in control animals. Based on the ability of AT1 receptor antagonists to block AngII effects in these mutant mice, the AT1b receptor appears to play a significant role in regulation of renal hemodynamics. The meaning of the inhibitory effect of PD 123319 on a receptor responsible for vasoconstriction remains elusive. Evidence to date is consistent with AngII causing renal vasoconstriction in part via a receptor distinct from known AT1 and AT2 receptors.

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