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Article

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Increased renal vasodilator prostanoids prevent hypertension in mice lacking the angiotensin subtype-2 receptor

Helmy M. Siragy,¹ Takaaki Senbonmatsu,² Toshihiro Ichiki,² Tadashi Inagami,² and Robert M. Carey¹

¹Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908, USA

²Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA

Address correspondence and reprint requests to: Robert M. Carey, Box 395, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, USA. Phone: (804) 924-5510; Fax: (804) 982-3626; E-mail: rmc4c@virginia.edu.

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The angiotensin subtype-1 (AT₁) receptor mediates renal prostaglandin E₂ (PGE₂) production, and pharmacological blockade of the angiotensin subtype-2 (AT₂) receptor potentiates the action of angiotensin II (Ang II) to increase PGE₂ levels. We investigated the role of the AT₂ receptor in prostaglandin metabolism in mice with targeted deletion of the AT₂ receptor gene. Mice lacking the AT₂ receptor (AT₂-null) had normal blood pressure that was slightly elevated compared with that of wild-type (WT) control mice. AT₂-null mice had higher renal interstitial fluid (RIF) 6-keto-PGF_{1α} (a stable hydrolysis product of prostacyclin [PGI₂]) and PGE₂ levels than did WT mice, and had similar increases in PGE₂ and 6-keto-PGF_{1α} in response to dietary sodium restriction and Ang II infusion. In contrast, AT₂-null mice had lower PGF_{2α} levels compared with WT mice during basal conditions and in response to dietary sodium restriction or infusion of Ang II. RIF cAMP was markedly higher in AT₂-null mice than in WT mice, both during basal conditions and during sodium restriction or Ang II infusion. AT₁ receptor blockade with losartan decreased PGE₂, PGI₂, and cAMP to levels observed in WT mice. To determine whether increased vasodilator prostanoids prevented hypertension in AT₂-null mice, we treated AT₂-null and WT mice with indomethacin for 14 days. PGI₂, PGE₂, and cAMP were markedly decreased in both WT and AT₂-null mice. Blood pressure increased to hypertensive levels in AT₂-null mice but was unchanged in WT. These results demonstrate that in the absence of the AT₂ receptor, increased vasodilator prostanoids protect against the development of hypertension.

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Introduction

The renin-angiotensin system constitutes a major hormonal cascade regulating fluid and electrolyte balance and blood pressure (BP), the principal effector of which is angiotensin II (Ang II) (1). The vast majority of the physiological effects of Ang II are mediated by the subtype-1 (AT₁) angiotensin receptor; the function of the subtype-2 (AT₂) receptor remains to be determined (2). AT₂ receptors are present in the kidney and are upregulated by sodium depletion and Ang II (3, 4).

Ang II stimulates production of prostacyclin (PGI₂) and prostaglandin E₂ (PGE₂) (5). The angiotensin receptor subtype mediating renal prostanoid formation is largely unknown. We have shown previously in the rat kidney that the AT₁ receptor mediates Ang II-induced PGE₂ production and that pharmacological blockade of the AT₂ receptor potentiates Ang II-induced PGE₂ levels (6). We also demonstrated that Ang II stimulates an increase in renal PGF_{2α} through an action at the AT₂ receptor (7).

This study was conducted to evaluate the role of the AT₂ receptor in prostaglandin metabolism and to investigate the mechanisms involving prostaglandins in maintaining normal BP. To address these issues, we used a novel microdialysis technique and mice with targeted

disruption of the AT₂ receptor gene to monitor changes in 6-keto-PGF_{1α} (a stable hydrolysis product of PGI₂), PGE₂, PGF_{2α}, and cAMP (the major second messenger of PGE₂ and PGI₂) in renal interstitial fluid (RIF) (5, 8).

Methods

Targeted disruption of the mouse AT₂ receptor gene. This procedure was performed as described (9). In brief, the coding region of embryonic stem cells (E14-1) in the third exon of the AT₂ receptor gene on the X chromosome was disrupted with a construct containing a neomycin-resistant expression cassette and herpes simplex virus thymidine-kinase expression cassette. The gene-disrupted cells were selected by G418 and ganciclovir, followed by Southern blot analysis to validate the desired gene disruption. Four targeted clones were obtained from 800 double-resistant colonies, 2 of which were injected into blastocysts derived from C57BL/6 mice, and the blastocysts were implanted into the uterus of pseudopregnant JCR mice. Of 34 chimeras, germ-line transmission occurred in 11 mice. Heterozygous female mice and wild-type (WT) male mice were mated to generate hemizygous male mice. Homozygous female mice were generated by mating heterozygous females with hemizygous males. The presence of a homozygous mutated AT₂ receptor

gene was confirmed by Southern blot analysis.

Homozygous female AT₂-null mice (*agtr2*^{-/-}) and their WT counterparts (*agtr2*^{+/+}) were generated from the littermates produced from the third backcross of heterozygous females (*agtr2*^{+/-}) to C57BL/6 males (*agtr2*^{+/-}), as follows. Heterozygous females (*agtr2*^{+/-}) and hemizygous males (*agtr2*^{+/-}) were crossed to obtain homozygous females (*agtr2*^{-/-}). From the same littermates, heterozygous females (*agtr2*^{+/-}) and WT males (*agtr2*^{+/+}) were crossed to obtain WT females (*agtr2*^{+/+}). Mice with the *agtr2*^{-/-} genotype were used as AT₂-null mice. Those with the *agtr2*^{+/+} genotypes were used as WT. The genotype of each individual mouse was confirmed by Southern blotting of DNA from the tail, as described (9).

Our AT₂-null mice were at the third backcross stage, which did not warrant the use of inbred C57BL/6 females as control. Therefore, we produced female *agtr2*^{+/+} and *agtr2*^{-/-} mice by mating littermates produced from the third backcross.

In vivo microdialysis technique. For the determination of RIF autacoids, we constructed a microdialysis probe using a modification of a technique described previously (6, 7, 10). Substances with a molecular mass greater than 10 kDa cannot cross into the dialysis probe. This molecular mass cutoff allows free passage of 6-keto-PGF_{1α}, PGE₂, PGF_{2α}, and cAMP. The dead volume of the dialysis tubing and outflow tube was 3.6 μL. The microdialysis probe was sterilized by a gas sterilization method. The rate of flow of dialysate was maintained at 3 μL/min.

In vitro microdialysis. RIF prostaglandin levels generally parallel, but are more sensitive than, urine prostaglandin excretion. In vitro best recoveries for renal autacoids were observed with a perfusion rate of 3 μL/min, and were 81% for 6-keto-PGF_{1α}, 63% for PGE₂, 60% for PGF_{2α}, and 84% for cAMP. We have demonstrated previously that negligible amounts of these substances stick to the polyethylene tubes (6).

Animal preparation. Experiments were conducted in 25 homozygous and 25 WT mice 12–16 weeks old. Mice were anesthetized with 80 mg/kg ketamine (Fort Dodge Laboratories, Fort Dodge, Iowa, USA) and 8 mg/kg xylazine (Bayer Corp., Animal Health Division, Shawnee, Kansas, USA), both administered intramuscularly. The right and left kidneys were exposed by a midline abdominal incision. Microdialysis probes were inserted into the kidneys of both the homozygous and WT mice. The renal capsule was penetrated with a 31-gauge needle that was tunneled into the outer renal cortex about 1 mm from the outer renal surface for 0.3 cm before it exited by penetrating the capsule again. The tip of the needle was inserted into the end of the dialysis probe, and the needle was pulled through with the dialysis tube until the dialysis fiber was situated in the renal cortex. The inflow and outflow tubes of the dialysis probes were tunneled subcutaneously through a bevel-tipped stainless steel tube and exteriorized near the intrascapular region. The exterior ends of the tubes were secured in place by suturing them to the skin at the exit site. The exteriorized portions of the tubes were placed in a stainless steel spring (to prevent the mice from damaging them).

To infuse Ang II or vehicle, an osmotic minipump (model 1007D; Alza Corp., Palo Alto, California, USA) was implanted in the subcutaneous space in the interscapular area, and Ang II or vehicle was infused subcutaneously. No tissue necrosis was observed with Ang II infusion.

Mice were housed under controlled conditions (temperature 21 ± 1 °C, humidity 60 ± 10%, lighting for 8–20 hours). Experiments were initiated at the same time each day to avoid diurnal variation of the measured body weight, systolic blood pressure (SBP), or RIF mediators. For collection of RIF, the inflow tube was connected to a gas-tight syringe that was filled with lactated Ringer's solution and perfused at 3 μL/min. The effluent was collected from the outflow tube for 30-minute sample periods in nonheparinized plastic tubes and stored at -80 °C until measured for PGE₂, PGF_{2α}, and cAMP. The known PGI₂- and cAMP-generating and -degrading enzymes (mol wt = 34,000–150,000) do not cross the dialysis membrane because of their size. A histological examination of the renal tissue 6 weeks after insertion of the dialysis probe did not show any fibrosis or scarring (6).

Urine was collected in special metabolic cages designed for individual mice. Urine was collected by a closed system that prevents evaporation or fecal contamination.

BP measurements. SBP was measured in the tail artery in homozygous and WT mice under restraint using an automated sphygmomanometer (model 679; IITC/Life Sciences Instruments, Woodland, California, USA) after a 7-day training period. BPs were recorded at 10-minute intervals for 30 minutes each morning during the study period (model 179 Apollo Recorder; Life Sciences Instruments), and values were averaged each day.

Analytical methods. Urinary sodium concentrations were measured with a Nova analyzer (Nova Biomedical, Waltham, Massachusetts, USA). PGE₂, PGF_{2α}, and 6-keto-PGF_{1α} were measured by an enzymatic immunoassay (Cayman Chemical, Ann Arbor, Michigan, USA). The sensitivities and specificities of the assay for PGE₂ were 114 pg/mL and 100%, respectively; for PGF_{2α}, 14.2 pg/mL and 100%, respectively; and for 6-keto-PGF_{1α}, 11 pg/mL and 100%, respectively. The intra- and interassay coefficients of variation were less than 10% for each assay. cAMP was measured by enzyme immunoassay (Cayman Chemical) with an assay sensitivity of 20 fmol and specificity of 100%.

Effects of dietary sodium restriction on BP, sodium excretion, and RIF mediators. Homozygous (*n* = 10) and WT (*n* = 10) mice were placed in metabolic cages. Baseline BPs and heart rates were measured, and a baseline 24-hour urine collection was obtained for calculation of urine flow rate (V) and sodium excretion (U_{Na}V). RIF samples were obtained for PGE₂, PGF_{2α}, 6-keto-PGF_{1α}, and cAMP (experimental day 1) while mice were consuming a normal sodium diet (0.28% NaCl; BioServe Biotechnologies Inc., Frenchtown, New Jersey, USA). After experimental day 1, mice were placed on a low-sodium diet (0.04% NaCl) for 7 days. On the seventh day of low sodium intake, the study was repeated as already outlined here.

Effects of chronic Ang II infusion on BP, sodium excretion, and RIF autacoids. Homozygous (*n* = 10) and WT (*n* = 10) mice were placed in metabolic cages on normal sodium intake for 10 days. On experimental day 1, a baseline

Table 1BP and urinary volume and sodium excretion in AT₂-null and WT mice

| | Wild-type | | | AT ₂ -null | | |
|-------------------------------------|--------------|---------------------------|-----------------|---------------------------|---------------------------|---------------------------|
| | Control | Sodium restriction | Ang II infusion | Control | Sodium restriction | ANG II infusion |
| Blood pressure (mmHg) | 104.8 ± 0.54 | 108.5 ± 1.8 | 107.1 ± 0.13 | 116.6 ^A ± 0.54 | 115.2 ± 0.8 | 196.6 ^B ± 1.2 |
| Urine volume (mL/24 h) | 1.4 ± 0.01 | 1.471 ± 0.23 | 1.19 ± 0.002 | 2.2 ± 0.05 | 2.3 ± 0.05 | 0.49 ^B ± 0.01 |
| Urinary sodium excretion (mEq/24 h) | 0.28 ± 0.02 | 0.02 ^C ± 0.045 | 0.25 ± 0.01 | 0.6 ± 0.015 | 0.05 ^B ± 0.002 | 0.07 ^C ± 0.003 |

^A = $P < 0.001$ from WT. ^B $P < 0.0002$ from control. ^C $P < 0.0001$ from control.

24-hour urine collection was obtained for calculation of V and U_{Na}V, and RIF samples for PGE₂, PGF_{2α}, 6-keto-PGF_{1α}, and cAMP were obtained. At 0800 hours on study experimental day 2, a subcutaneous infusion of Ang II (4 pmol/kg/min) or vehicle was initiated and continued for 6 days (experimental days 2–7) using the osmotic minipump. BP, heart rate, V, and U_{Na}V were monitored daily. On experimental day 7, RIF samples for autacoids were again obtained. At 0800 hours on experimental day 8, the infusion of Ang II was discontinued, and a vehicle infusion was substituted for 4 additional days (experimental days 8–11) while BP, HR, V, and U_{Na}V measurements were continued.

Effects of indomethacin on BP, sodium excretion, and RIF mediators. To determine whether the absence of a large rise in baseline BP in AT₂-null mice was due to an increase in renal PGE₂, we administered indomethacin, a cyclooxygenase inhibitor, to homozygous ($n = 10$) and WT ($n = 10$) mice during normal sodium intake. After baseline RIF measurements on control day 0, as already described here, mice were given indomethacin (5 mg/kg/d) or vehicle intraperitoneally for 14 experimental days. BP was measured daily. At the end of this time, the measurements were repeated. No gastrointestinal toxicity due to indomethacin was observed.

Effects of AT₁ receptor blockade on RIF autacoids in AT₂-null mice. To determine the mechanism of the increase in renal PGE₂, 6-keto-PGF_{1α}, and cAMP in AT₂-null mice, we administered a bolus dose of 10 mg/kg of the AT₁ receptor antagonist losartan via tail vein to conscious AT₂-null mice ($n = 5$) with an indwelling renal microdialysis probe. Two hours later, RIF was obtained over a 1-hour collection period for PGE₂, 6-keto-PGF_{1α}, and cAMP.

Effects of AT₂ receptor blockade on RIF autacoids in WT. To confirm the mechanism of the reduction in PGF_{2α} in AT₂-null mice, we administered the AT₂ receptor antagonist PD123319 (PD; 50 μg/kg/min) to WT mice ($n = 5$) by osmotic minipump for 5 consecutive days. RIF samples were collected on day 1 after initiation of a vehicle infusion or infusion of PD with the animals on a normal sodium diet (0.31% sodium), after which they were placed on a low-sodium diet (0.08% sodium) for 4 days during vehicle or PD infusion. RIF PGE₂ and PGF_{2α} were again measured on day 5 of the low-sodium diet in the presence of PD or vehicle.

Statistical analysis. Comparisons between normal and low sodium intake, and between Ang II or

indomethacin and vehicle, were estimated by repeated-measures ANOVA, using the General Linear Models procedure of the Statistical Analysis System (Virginia Polytechnic and State University, Blacksburg, Virginia, USA). Multiple comparisons of individual pairs of effect means were conducted by least-square means pooled variance. Data are expressed as mean ± SEM. Statistical significance was identified at $P < 0.05$.

Results

BP, V, and U_{Na}V responses to dietary sodium restriction and Ang II infusion. BP was slightly but significantly higher in AT₂-null mice ($n = 10$) than in WT mice ($n = 10$) at baseline (Table 1). Sodium restriction did not alter BP in either AT₂-null or WT mice. Ang II infusion increased BP significantly in AT₂-null mice but not in WT mice. V and U_{Na}V were similar in AT₂-null and WT mice. In response to sodium restriction, V was unchanged but U_{Na}V was significantly reduced in both WT and AT₂-null mice. Ang II infusion decreased V and U_{Na}V significantly in AT₂-null mice but not in WT mice.

RIF PGE₂, PGF_{2α}, 6-keto-PGF_{1α}, and cAMP responses to dietary sodium restriction. Figure 1a depicts PGE₂ levels during normal sodium intake and after 7 days of dietary sodium restriction. During normal sodium intake, PGE₂ was significantly increased in AT₂-null mice compared with WT mice. Dietary sodium restriction increased PGE₂ both in AT₂-null and WT mice. In sodium-restricted animals, PGE₂ levels in AT₂-null mice were more than 2-fold higher than in WT mice. Figure 1b demonstrates PGF_{2α} levels. PGF_{2α} was significantly lower in AT₂-null mice than in WT mice during normal sodium intake. Dietary sodium restriction increased PGF_{2α} in WT mice but significantly decreased PGF_{2α} in AT₂-null mice. In sodium-restricted animals, PGF_{2α} levels were more than 10-fold higher in WT mice than in AT₂-null mice. Figure 1c shows RIF levels of 6-keto-PGF_{1α}. Basal levels of 6-keto-PGF_{1α} were higher in AT₂-null mice than in WT mice. In both WT and AT₂-null mice, 6-keto-PGF_{1α} increased significantly in response to sodium restriction. There was no significant difference in the incremental change to sodium restriction between WT and AT₂-null mice. AT₂-null mice had approximately 26-fold higher cAMP levels (Figure 1d) than did WT mice during normal sodium intake. Low sodium intake resulted in no significant change in cAMP in either AT₂-null or WT mice.

RIF PGE₂, PGF_{2α}, 6-keto-PGF_{1α}, and cAMP responses to chronic Ang II infusion. AT₂-null mice had higher PGE₂

levels (Figure 2a) than did WT mice during vehicle infusion. Ang II infusion increased PGE₂ in both WT and AT₂-null mice, the increase being significantly greater in WT mice. After Ang II infusion, AT₂-null mice had PGE₂ levels similar to those in WT mice. AT₂-null mice had lower PGF_{2α} levels (Figure 2b) than did WT mice during vehicle infusion. In WT mice, PGF_{2α} was increased approximately 4-fold by chronic Ang II infusion, whereas in AT₂-null mice, there was no significant change in PGF_{2α} levels in response to Ang II. Responses of 6-keto-PGF_{1α} to exogenous Ang II are shown in Figure 2c. Basal control levels of 6-keto-PGF_{1α} were higher in AT₂-null mice than in WT mice. Both WT and AT₂-null mice had significant increases in 6-keto-PGF_{1α} in response to Ang II, and there was no difference in these responses between the two. cAMP values (Figure 2d) were significantly higher in AT₂-null mice than in WT mice, both basally and in response to Ang II. Ang II did not significantly alter cAMP in either WT or AT₂-null mice.

BP responses to indomethacin. In AT₂-null mice, BP increased progressively and dramatically well into the hypertensive range in response to intraperitoneally administered indomethacin (5 mg/kg/d) (Figure 3).

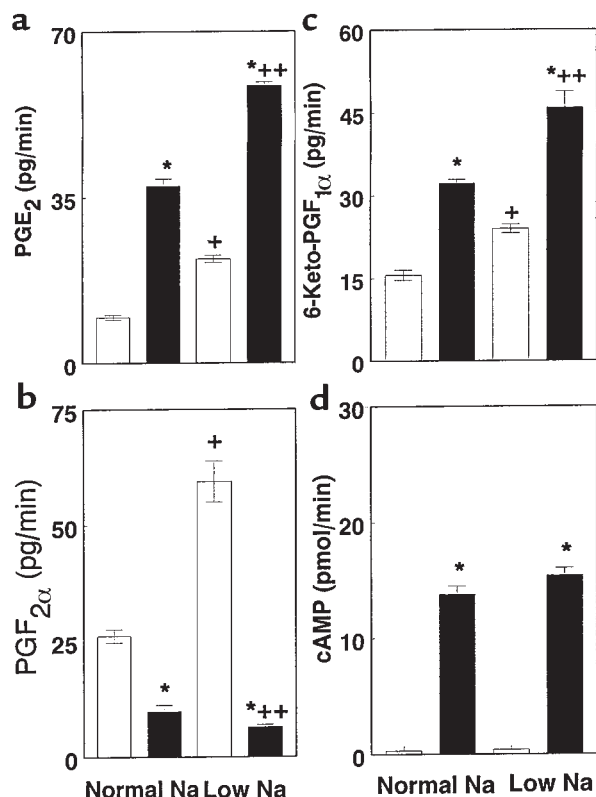


Figure 1
RIF PGE₂ (a), PGF_{2α} (b), 6-keto-PGF_{1α} (c), and cAMP (d) in mice ($n = 10$) lacking the AT₂ angiotensin receptor (filled bars) and in WT mice ($n = 10$; open bars), on normal or low dietary sodium (Na) intake. (a) * $P < 0.0005$ vs. WT; + $P < 0.005$, ++ $P < 0.001$ vs. normal sodium intake. (b) * $P < 0.001$ vs. WT; + $P < 0.05$, ++ $P < 0.001$ vs. normal sodium intake. (c) * $P < 0.001$ vs. WT; + $P < 0.01$, ++ $P < 0.001$ vs. normal sodium intake. (d) * $P < 0.0005$ vs. WT.

There was no change in BP on any day of the vehicle time control. BP before vehicle administration was 102 ± 5 mmHg and was 103 ± 2 mmHg after 14 days of vehicle administration. There was no change in BP in WT mice in response to indomethacin (Figure 3).

RIF PGE₂, PGF_{2α}, and cAMP responses to chronic indomethacin administration. PGE₂ (Figure 4a) values were about 3.5-fold higher in AT₂-null mice than in WT mice on day 0 before indomethacin administration. In response to 14 consecutive days of indomethacin administration, PGE₂ was significantly decreased in both WT and AT₂-null mice. PGE₂ values were not significantly different in WT and AT₂-null mice after 14 days of indomethacin administration. PGF_{2α} levels (Figure 4b) were more than 3-fold lower in AT₂-null mice than in WT mice at baseline. Indomethacin lowered PGF_{2α} both in WT and AT₂-null mice. In indomethacin-treated animals, PGF_{2α} was significantly lower in AT₂-null mice than in WT mice. Indomethacin decreased 6-keto-PGF_{1α} levels significantly in both WT and AT₂-null mice, such that levels were not significantly different between the two (Figure 4c). cAMP levels (Figure 4d) in WT mice were low and were unchanged by indomethacin. In AT₂-null mice, cAMP levels were relatively high at baseline and were markedly reduced.

RIF PGE₂, 6-keto-PGF_{1α}, and cAMP responses to AT₁ receptor blockade in AT₂-null mice. As shown in Figure 5, control values for PGE₂, 6-keto-PGF_{1α}, and cAMP in AT₂-null mice were 39 ± 7 , 39 ± 4 , and 16 ± 3 pg/min, respectively. In response to losartan, PGE₂ decreased ($P < 0.0001$) to less than one third of control values and to a value similar to that of WT mice. Similarly, 6-keto-PGF_{1α} decreased to about 40% of control values ($P < 0.0001$). Losartan caused a parallel reduction of cAMP ($P < 0.0001$) to values similar to those of WT mice.

RIF PGE₂ and PGF_{2α} responses to AT₂ receptor blockade in sodium-restricted WT mice. Figure 6 depicts the incremental increase in PGE₂ and PGF_{2α} in response to dietary sodium restriction for 5 days in the presence or absence of continuous intravenous infusion of PD. Sodium restriction increased both PGE₂ and PGF_{2α} in mice receiving only vehicle infusion. AT₂ receptor blockade with PD further increased PGE₂ but decreased the PGF_{2α} response to sodium restriction (both $P < 0.01$).

Discussion

This study demonstrates that Ang II acts at the AT₁ receptor to stimulate both PGI₂ and PGE₂ production in the kidney. PGE₂ is the major renal prostanoid and has substantially greater vasodilator potency than PGI₂ in the rat kidney (5). PGI₂ is the major metabolite of arachidonic acid in vascular endothelial cells (6). The baseline increase in renal PGE₂ and 6-keto-PGF_{1α} in AT₂-null mice is the result of interruption of receptor cross-talk and the presence of “unopposed” AT₁ receptors in these mice, as AT₁ receptor blockade normalized PGE₂ and 6-keto-PGF_{1α} in AT₂-null mice. These observations suggest that the AT₂ receptor has a tonic inhibitory action on the production of PGI₂ and PGE₂, which is driven by the AT₁ receptor.

It is generally appreciated that Ang II can stimulate PGI₂ and PGE₂ production in nonrenal tissues. Studies

using isolated vascular smooth muscle cells and isolated perfused heart preparations have demonstrated that Ang II stimulates PGI₂ release through an interaction at AT₁ receptors (11–15). However, the angiotensin subtype receptor responsible for renal PGE₂ and PGI₂ formation has not been studied systematically. The results of the present study show that both renal PGI₂ and PGE₂ production are stimulated in parallel by either endogenous (sodium restriction) or exogenous Ang II.

PGI₂ and PGE₂ are both formed from the precursor PGH₂, which is a product of cyclooxygenase action on its substrate, arachidonic acid. PGF_{2α}, another major renal prostanoid, can also be formed directly from PGH₂ or, alternatively, by conversion from PGE₂ through the action of PGE₂ 9-ketoreductase (5). Prostaglandins are paracrine or autocrine substances, acting locally in the tissues in which they are formed (5). Because of their rapid degradation on passage across the lungs, these substances are not detectable in appreciable quantities in the circulation. However, prostanoids can be assayed and changes monitored in RIF with a high degree of sensitivity (6). PGE₂ and PGF_{2α} are produced in renal interstitial and collecting duct cells and in the endothelium of vasa recta, and they participate in the regulation of renal function (16, 17). The production of these autacoids is augmented by pressor hormones, including Ang II via stimulation of phospholipase A₂, to form arachidonic acid substrate from membrane phospholipids (5, 18). Ang II stimulates the endothelial release of arachidonic acid metabolites (19–21). We have shown previously in the rat that Ang II stimulates renal PGE₂ production through the AT₁ receptor (6). Our present results are consistent with earlier observations in the normal rat kidney that conversion of PGE₂ to PGF_{2α} by 9-ketoreductase is enhanced by sodium depletion (22, 23).

An important finding of the present study is that Ang II acts at the AT₂ receptor to stimulate PGF_{2α}, probably by conversion from PGE₂. At baseline, mice lacking the AT₂ receptor had increased RIF levels of PGE₂ and low levels of PGF_{2α}. In WT mice, both sodium restriction (endogenous Ang II) and chronic infusion of a small quantity of exogenous Ang II increased 6-keto-PGF_{1α}, PGE₂, and PGF_{2α}. Similarly, in AT₂-null mice, sodium restriction and chronic Ang II infusion increased 6-keto-PGF_{1α} and PGE₂. In marked contrast to WT mice, however, in AT₂-null mice, both exogenous and endogenous Ang II failed to increase PGF_{2α}. These results were

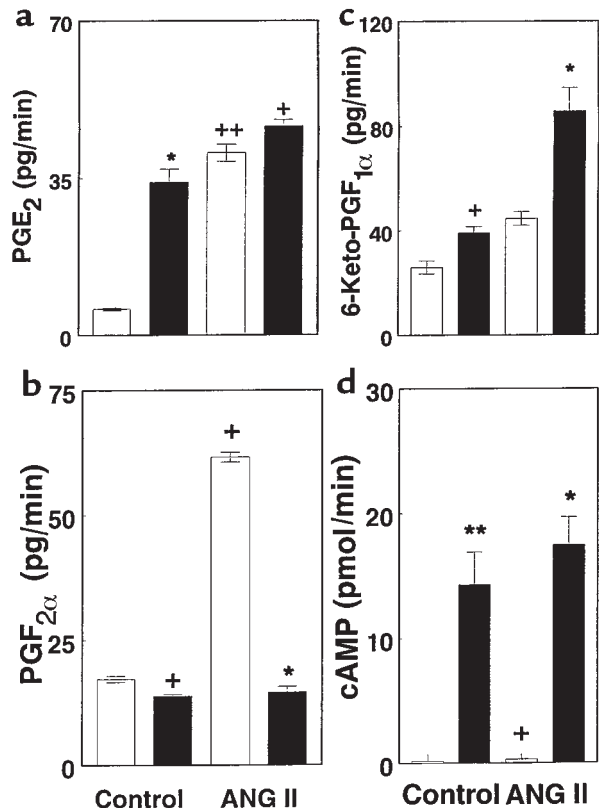


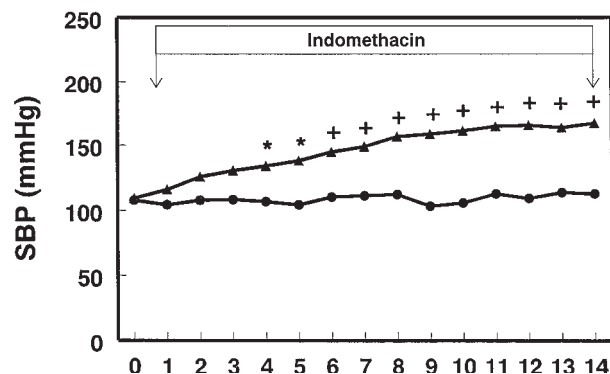
Figure 2

RIF PGE₂ (a), PGF_{2α} (b), 6-keto-PGF_{1α} (c), and cAMP (d) in mice (*n* = 10) lacking the AT₂ angiotensin receptor (filled bars) and in WT mice (*n* = 10; open bars) during the vehicle control period (Control) and after 7 days of continuous infusion of Ang II. (a) **P* < 0.01 vs. WT; **P* < 0.05, ***P* < 0.0005 vs. control. (b) **P* < 0.001, ***P* < 0.0001 vs. WT; **P* < 0.0001 vs. control. (c) **P* < 0.0005 vs. WT; **P* < 0.05 vs. control. (d) **P* < 0.01, ***P* < 0.001 vs. WT; **P* < 0.002 vs. control.

confirmed by experiments showing that sodium restriction increased PGE₂, PGI₂, and PGF_{2α}, and by separate experiments showing that AT₂ receptor blockade with PD blocked the increase in PGE₂ in WT mice. Our results underscore the physiological importance of the AT₂ receptor in the formation of PGF_{2α}, most likely through conversion from PGE₂ by 9-ketoreductase. Our data, however, do not permit determination of the effects, if any, of the AT₂ receptor on PGH₂ metabolism. Our data demonstrate that the increase in basal PGE₂

Figure 3

SBP of mice (*n* = 10) lacking the AT₂ angiotensin receptor (triangles) and of WT mice (*n* = 10, circles) during vehicle infusion (day 0) and during continuous intraperitoneal infusion of indomethacin (5 mg/kg/d) for 14 days. **P* < 0.001 vs. day 0 and WT. **P* < 0.0001 vs. day 0 and WT.



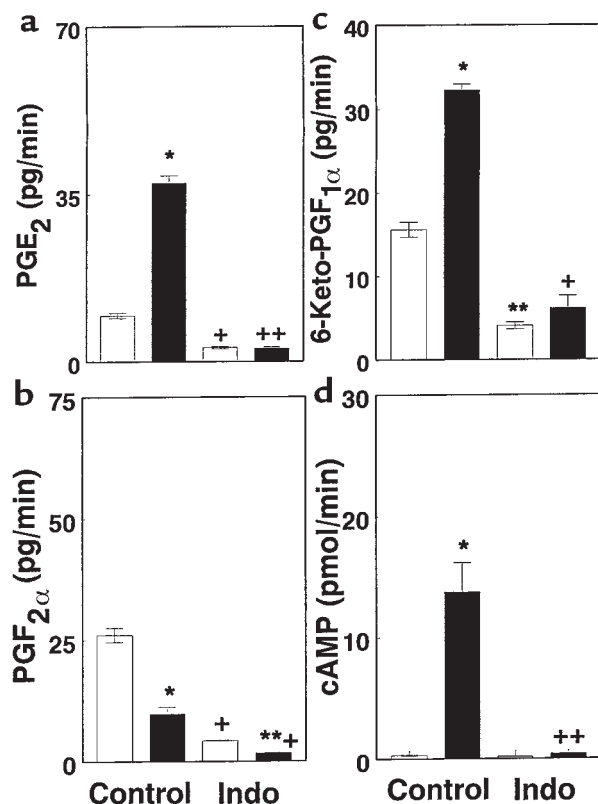


Figure 4
RIF PGE₂ (a), PGF_{2α} (b), 6-keto-PGF_{1α} (c), and cAMP (d) in mice (*n* = 10) lacking the AT₂ angiotensin receptor (filled bars) and in WT mice (*n* = 10; open bars) during the vehicle control period (Control) and after 14 days of indomethacin administration (5 mg/kg/d intraperitoneally). (a) **P* < 0.0005 vs. WT; **P* < 0.005, ***P* < 0.0005 vs. control. (b) **P* < 0.001, ***P* < 0.0001 vs. WT; **P* < 0.0001 vs. control. (c) **P* < 0.001 vs. WT; **P* < 0.0001, ***P* < 0.0001 vs. control. (d) **P* < 0.001 vs. WT. ***P* < 0.0005 vs. control.

in AT₂-null mice is largely related to absence of tonic inhibition of the AT₂ receptor on AT₁ receptor-mediated PGE₂ production.

The interaction between the renin-angiotensin system and renal eicosanoids is fundamental to the regulation of body fluid and sodium homeostasis (8). PGI₂ and PGE₂ stimulate, whereas PGF_{2α} inhibits, renin secretion (24). AT₂ receptor-mediated stimulation of PGF_{2α} formation, and its inhibitory effect on renin secretion, is in agreement with recent findings that the AT₂ receptor may indirectly mediate vasodilation (9). PGE₂ inhibits sodium reabsorption in the cortical and medullary collecting ducts and leads to natriuresis (25). Sodium depletion increases renal Ang II formation (26), and it is likely that the resulting increase in conversion of PGE₂ to PGF_{2α} is related to increased Ang II formation and stimulation of the AT₂ receptor. The decrease in sodium excretion during sodium restriction is likely mediated by AT₁ receptors in renal tubule cells in both WT and AT₂-null mice.

The tonic inhibitory action of the AT₂ receptor on AT₁ receptor-mediated PGI₂ and PGE₂ production may be due to several possible factors. (a) Nitric oxide,

which is increased by AT₂ receptors, has been described to inhibit cyclooxygenase under some conditions (27, 28). (b) cGMP, also increased by AT₂ receptors, may affect prostaglandin metabolism (28). (c) Bradykinin, also increased by AT₂ receptors, can stimulate the production of nitric oxide and eicosanoids in the intact kidney (29). (d) The AT₂ receptor may stimulate epoxide production through the cytochrome P₄₅₀ system, which may compete with arachidonic acid for metabolism by cyclooxygenase, resulting in inhibition of PGI₂ and PGE₂ formation (30, 31). These possibilities will need to be resolved in future studies.

PGF_{2α}, the product of PGE₂ metabolism by 9-ketoreductase, binds mainly to the recently cloned FP receptor, resulting in a rise in intracellular calcium (32). The FP receptor is thought to mediate vasoconstriction. PGF_{2α} also can bind to EP₁ and EP₃ receptors, both of which also mediate vasoconstriction (32). In AT₂-null mice, the decrease in PGF_{2α} may contribute to the absence of hypertension by reducing peripheral and/or renal vasoconstriction.

PGE₂ has been shown to stimulate adenylyl cyclase via the EP₂ and EP₄ receptors with the generation of cAMP, which acts as its second messenger (8, 32). These 2 receptors mediate vascular smooth muscle relaxation. cAMP is extruded from target cells by a prostanoid-sensitive membrane transporter and can be measured in the RIF. Our data demonstrate that cAMP levels were high in AT₂-null mice (PGE₂ and PGI₂ levels also were elevated) compared with WT mice. An unanticipated finding was the failure of cAMP to increase in WT mice in response to sodium restriction or Ang II in proportion to the increase in PGE₂. The parallel reduction of PGE₂, 6-keto-PGF_{1α}, and cAMP by indomethacin suggests, however, that PGE₂ and/or PGI₂ are very likely responsible for elevated cAMP in AT₂-null mice. Failure of cAMP to increase substantially in response to sodium restriction or Ang II in AT₂-null mice may indicate maximum stimulation of cAMP production in the basal state.

Both PGE₂ and PGI₂ are vasodilators, and excessive vasodilator prostanoid accumulation in the absence of the AT₂ receptor may account for the absence of hypertension in this model. To test this hypothesis, we administered the cyclooxygenase inhibitor indomethacin and monitored BP. In response to indomethacin, which decreased renal 6-keto-PGF_{1α}, PGE₂, and PGF_{2α} to low levels, BP steadily rose into the hypertensive range. These results suggest that increased PGI₂ and PGE₂ in AT₂-null mice prevented the increase in BP that otherwise would have occurred, most likely owing to a reduction of other vasodilators such as bradykinin and/or nitric oxide, which are stimulated via the AT₂ receptor (6). It is also possible that in addition to decreasing vasodilator prostanoid production, indomethacin may have selectively inhibited vasodilator prostanoid signal transduction in target cells, as has been demonstrated in the cerebral microcirculation (33). Therefore, the action of indomethacin to increase BP could be related to inhibition of both vasodilator prostanoid synthesis and action.

There are several limitations to the present study. (a) We were unable to measure plasma renin activity or Ang II concentrations because volume depletion might have

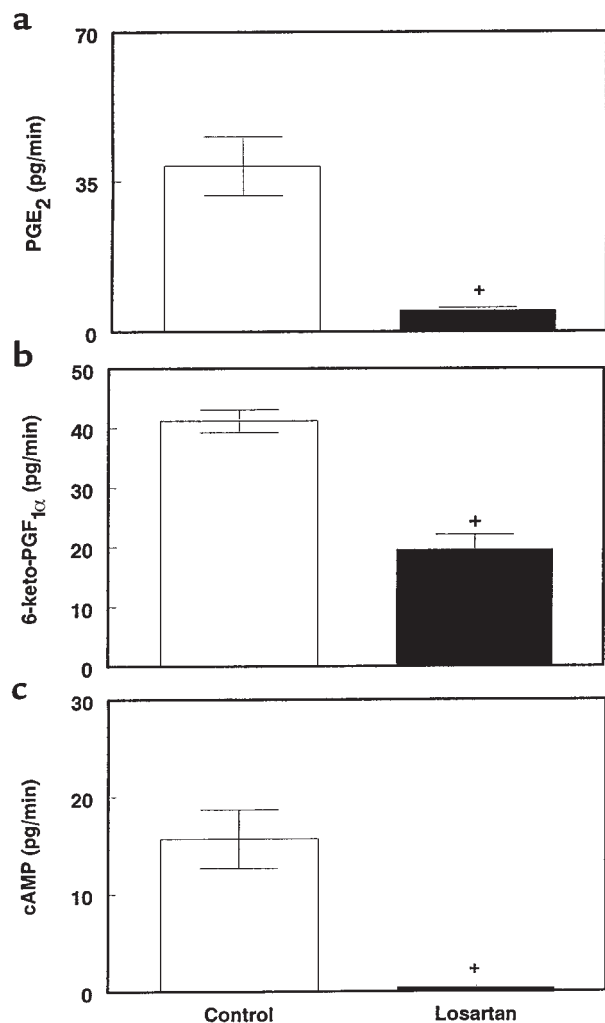


Figure 5
RIF PGE₂ (a), 6-keto-PGF_{1α} (b), and cAMP (c) in WT mice ($n = 5$) before and 2 hours after intravenous losartan administration (10 mg/kg). * $P < 0.0001$ vs. control.

altered RIF autacoid levels. (b) Although we were able to monitor PGE₂ and PGF_{2α} levels, we were not able to measure the activity of PGE₂ 9-ketoreductase directly. (c) Indomethacin has been reported to inhibit enzyme systems and cell signaling processes other than cyclooxygenase activity, including cAMP- and cGMP-phosphodiesterases, cAMP-dependent protein kinases and endogenous phosphorylation, active Ca²⁺ transport, and adenosine uptake (33). However, none of these potential actions of indomethacin could account for the ability of indomethacin to raise systemic BP or to decrease prostanoid or cAMP levels.

In summary, we have demonstrated that absence of the AT₂ receptor results in increased PGI₂ and PGE₂ and decreased PGF_{2α} levels in the kidney, both basally and in response to Ang II. These findings establish a physiological role of the AT₂ receptor in renal prostanoid production and metabolism. We also demonstrated that the renal vasodilator prostanoids PGE₂ and PGI₂ are stimulated via the AT₁ receptor, pro-

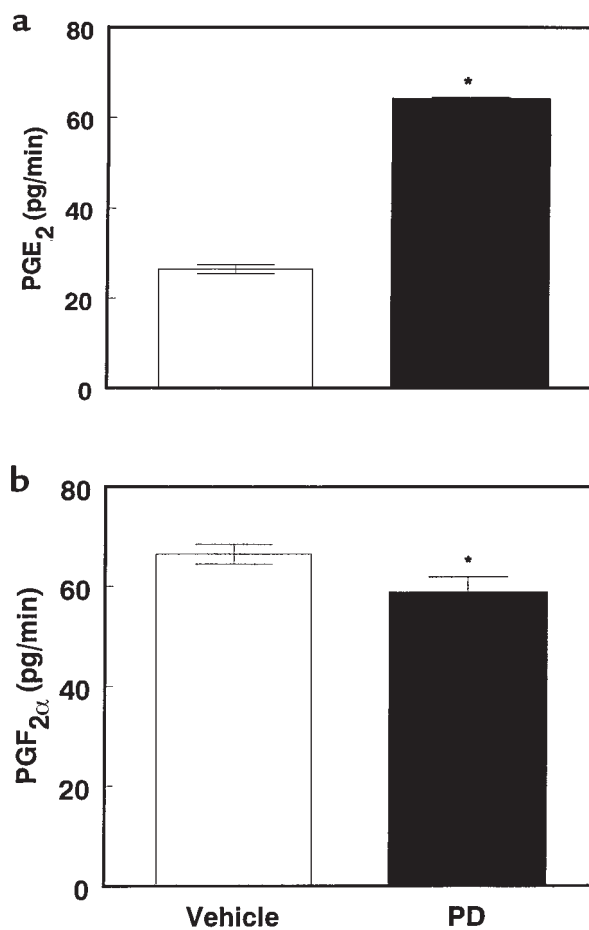


Figure 6
Incremental increase in RIF PGE₂ (a) and PGF_{2α} (b) in WT mice ($n = 5$) in response to 5 days of dietary sodium restriction in the presence or absence of the AT₂ receptor antagonist PD123319 (PD; 50 μg/kg/min). * $P < 0.001$ vs. vehicle control.

viding counterregulatory vasodilation in opposition to the vasoconstrictor action of Ang II. Most importantly, we demonstrated that in the absence of the AT₂ receptor, increased levels of these vasodilator prostanoids prevent the development of hypertension.

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