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

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The angiotensin subtype-1 (AT_1) receptor mediates renal prostaglandin E_2 (PGE_2) production, and pharmacological blockade of the angiotensin subtype-2 (AT_2) receptor potentiates the action of angiotensin II (Ang II) to increase PGE_2 levels. We investigated the role of the AT_2 receptor in prostaglandin metabolism in mice with targeted deletion of the AT_2 receptor gene. Mice lacking the AT_2 receptor (AT_2-null) had normal blood pressure that was slightly elevated compared with that of wild-type (WT) control mice. AT_2-null mice had higher renal interstitial fluid (RIF) 6-keto-$PGF_{1\alpha}$ (a stable hydrolysis product of prostacyclin [PGI_2]) and PGE_2 levels than did WT mice, and had similar increases in PGE_2 and 6-keto-$PGF_{1\alpha}$ in response to dietary sodium restriction and Ang II infusion. In contrast, AT_2-null mice had lower $PGF_{2\alpha}$ 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 AT_2-null mice than in WT mice, both during basal conditions and during sodium restriction or Ang II infusion. AT_1 receptor blockade with losartan decreased PGE_2, PGI_2, and cAMP to levels observed in WT mice. To determine whether increased vasodilator prostanoids prevented hypertension in AT_2-null mice, we treated AT_2-null and WT mice with indomethacin for 14 days. PGI_2, PGE_2, and cAMP were markedly decreased in both WT and AT_2-null mice. Blood pressure increased to hypertensive levels in AT_2-null mice but was unchanged in WT. These results demonstrate that in the absence of the AT_2 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 (AT_1) angiotensin receptor; the function of the subtype-2 (AT_2) receptor remains to be determined (2). AT_2 receptors are present in the kidney and are upregulated by sodium depletion and Ang II (3, 4).

Ang II stimulates production of prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) (5). The angiotensin receptor subtype mediating renal prostanoid formation is largely unknown. We have shown previously in the rat kidney that the AT_1 receptor mediates Ang II–induced PGE_2 production and that pharmacological blockade of the AT_2 receptor potentiates Ang II–induced PGE_2 levels (6). We also demonstrated that Ang II stimulates an increase in renal $PGF_{2\alpha}$ through an action at the AT_2 receptor (7).

This study was conducted to evaluate the role of the AT_2 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 AT_2 receptor gene to monitor changes in 6-keto-$PGF_{1\alpha}$ (a stable hydrolysis product of PGI_2), PGE_2, $PGF_{2\alpha}$, and cAMP (the major second messenger of PGE_2 and PGI_2) in renal interstitial fluid (RIF) (5, 8).

Methods

Targeted disruption of the mouse AT_2 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 AT_2 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 AT_2 receptor

gene was confirmed by Southern blot analysis.

Homozygous female AT_2-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 ($agtr2^{+/Y}$), as follows. Heterozygous females ($agtr2^{+/-}$) and hemizygous males ($agtr2^{-/Y}$) were crossed to obtain homozygous females ($agtr2^{-/-}$). From the same littermates, heterozygous females ($agtr2^{+/-}$) and WT males ($agtr2^{+/Y}$) were crossed to obtain WT females ($agtr2^{+/+}$). Mice with the $agtr2^{-/-}$ genotype were used as AT_2-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 AT_2-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 $agtr^{-/-}$ 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-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, 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-$PGF_{1\alpha}$, 63% for PGE_2, 60% for $PGF_{2\alpha}$, 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 PGE_2, $PGF_{2\alpha}$, and cAMP. The known PGI_2- 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). PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ were measured by an enzymatic immunoassay (Cayman Chemical, Ann Arbor, Michigan, USA). The sensitivities and specificities of the assay for PGE_2 were 114 pg/mL and 100%, respectively; for $PGF_{2\alpha}$, 14.2 pg/mL and 100%, respectively; and for 6-keto-$PGF_{1\alpha}$, 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 PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, 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 1
BP and urinary volume and sodium excretion in AT_2-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 PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, 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 AT_2-null mice was due to an increase in renal PGE_2, 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 AT_1 receptor blockade on RIF autacoids in AT_2-null mice. To determine the mechanism of the increase in renal PGE_2, 6-keto-$PGF_{1\alpha}$, and cAMP in AT_2-null mice, we administered a bolus dose of 10 mg/kg of the AT_1 receptor antagonist losartan via tail vein to conscious AT_2-null mice (n = 5) with an indwelling renal microdialysis probe. Two hours later, RIF was obtained over a 1-hour collection period for PGE_2, 6-keto-$PGF_{1\alpha}$, and cAMP.

Effects of AT_2 receptor blockade on RIF autacoids in WT. To confirm the mechanism of the reduction in $PGF_{2\alpha}$ in AT_2-null mice, we administered the AT_2 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 PGE_2 and $PGF_{2\alpha}$ 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 AT_2-null mice (n = 10) than in WT mice (n = 10) at baseline (Table 1). Sodium restriction did not alter BP in either AT_2-null or WT mice. Ang II infusion increased BP significantly in AT_2-null mice but not in WT mice. V and $U_{Na}V$ were similar in AT_2-null and WT mice. In response to sodium restriction, V was unchanged but $U_{Na}V$ was significantly reduced in both WT and AT_2-null mice. Ang II infusion decreased V and $U_{Na}V$ significantly in AT_2-null mice but not in WT mice.

RIF PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and cAMP responses to dietary sodium restriction. Figure 1a depicts PGE_2 levels during normal sodium intake and after 7 days of dietary sodium restriction. During normal sodium intake, PGE_2 was significantly increased in AT_2-null mice compared with WT mice. Dietary sodium restriction increased PGE_2 both in AT_2-null and WT mice. In sodium-restricted animals, PGE_2 levels in AT_2-null mice were more than 2-fold higher than in WT mice. Figure 1b demonstrates $PGF_{2\alpha}$ levels. $PGF_{2\alpha}$ was significantly lower in AT_2-null mice than in WT mice during normal sodium intake. Dietary sodium restriction increased $PGF_{2\alpha}$ in WT mice but significantly decreased $PGF_{2\alpha}$ in AT_2-null mice. In sodium-restricted animals, $PGF_{2\alpha}$ levels were more than 10-fold higher in WT mice than in AT_2-null mice. Figure 1c shows RIF levels of 6-keto-$PGF_{1\alpha}$. Basal levels of 6-keto-$PGF_{1\alpha}$ were higher in AT_2-null mice than in WT mice. In both WT and AT_2-null mice, 6-keto-$PGF_{1\alpha}$ increased significantly in response to sodium restriction. There was no significant difference in the incremental change to sodium restriction between WT and AT_2-null mice. AT_2-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 AT_2-null or WT mice.

RIF PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and cAMP responses to chronic Ang II infusion. AT_2-null mice had higher PGE_2

levels (Figure 2a) than did WT mice during vehicle infusion. Ang II infusion increased PGE_2 in both WT and AT_2-null mice, the increase being significantly greater in WT mice. After Ang II infusion, AT_2-null mice had PGE_2 levels similar to those in WT mice. AT_2-null mice had lower $PGF_{2\alpha}$ levels (Figure 2b) than did WT mice during vehicle infusion. In WT mice, $PGF_{2\alpha}$ was increased approximately 4-fold by chronic Ang II infusion, whereas in AT_2-null mice, there was no significant change in $PGF_{2\alpha}$ levels in response to Ang II. Responses of 6-keto-$PGF_{1\alpha}$ to exogenous Ang II are shown in Figure 2c. Basal control levels of 6-keto-$PGF_{1\alpha}$ were higher in AT_2-null mice than in WT mice. Both WT and AT_2-null mice had significant increases in 6-keto-$PGF_{1\alpha}$ in response to Ang II, and there was no difference in these responses between the two. cAMP values (Figure 2d) were significantly higher in AT_2-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 AT_2-null mice.

BP responses to indomethacin. In AT_2-null mice, BP increased progressively and dramatically well into the hypertensive range in response to intraperitoneally administered indomethacin (5 mg/kg/d) (Figure 3). 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 PGE_2, $PGF_{2\alpha}$, and cAMP responses to chronic indomethacin administration. PGE_2 (Figure 4a) values were about 3.5-fold higher in AT_2-null mice than in WT mice on day 0 before indomethacin administration. In response to 14 consecutive days of indomethacin administration, PGE_2 was significantly decreased in both WT and AT_2-null mice. PGE_2 values were not significantly different in WT and AT_2-null mice after 14 days of indomethacin administration. $PGF_{2\alpha}$ levels (Figure 4b) were more than 3-fold lower in AT_2-null mice than in WT mice at baseline. Indomethacin lowered $PGF_{2\alpha}$ both in WT and AT_2-null mice. In indomethacin-treated animals, $PGF_{2\alpha}$ was significantly lower in AT_2-null mice than in WT mice. Indomethacin decreased 6-keto-$PGF_{1\alpha}$ levels significantly in both WT and AT_2-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 AT_2-null mice, cAMP levels were relatively high at baseline and were markedly reduced.

RIF PGE_2, 6-keto-$PGF_{1\alpha}$, and cAMP responses to AT_1 receptor blockade in AT_2-null mice. As shown in Figure 5, control values for PGE_2, 6-keto-$PGF_{1\alpha}$, and cAMP in AT_2-null mice were 39 ± 7, 39 ± 4, and 16 ± 3 pg/min, respectively. In response to losartan, PGE_2 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-$PGF_{1\alpha}$ 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 PGE_2 and $PGF_{2\alpha}$ responses to AT_2 receptor blockade in sodium-restricted WT mice. Figure 6 depicts the incremental increase in PGE_2 and $PGF_{2\alpha}$ in response to dietary sodium restriction for 5 days in the presence or absence of continuous intravenous infusion of PD. Sodium restriction increased both PGE_2 and $PGF_{2\alpha}$ in mice receiving only vehicle infusion. AT_2 receptor blockade with PD further increased PGE_2 but decreased the $PGF_{2\alpha}$ response to sodium restriction (both $P < 0.01$).

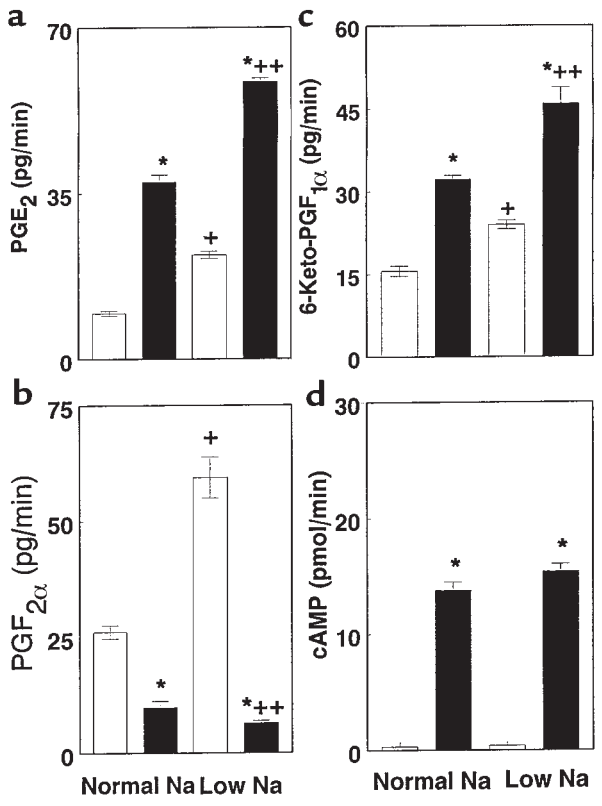

Figure 1
RIF PGE_2 (**a**), $PGF_{2\alpha}$ (**b**), 6-keto-$PGF_{1\alpha}$ (**c**), and cAMP (**d**) in mice ($n = 10$) lacking the AT_2 angiotensin receptor (filled bars) and in WT mice ($n = 10$; open bars), on normal or low dietary sodium (Na) intake. (**a**) $^*P < 0.0005$ vs. WT; $^+P < 0.005$, $^{++}P < 0.001$ 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.

Discussion

This study demonstrates that Ang II acts at the AT_1 receptor to stimulate both PGI_2 and PGE_2 production in the kidney. PGE_2 is the major renal prostanoid and has substantially greater vasodilator potency than PGI_2 in the rat kidney (5). PGI_2 is the major metabolite of arachidonic acid in vascular endothelial cells (6). The baseline increase in renal PGE_2 and 6-keto-$PGF_{1\alpha}$ in AT_2-null mice is the result of interruption of receptor cross-talk and the presence of "unopposed" AT_1 receptors in these mice, as AT_1 receptor blockade normalized PGE_2 and 6-keto-$PGF_{1\alpha}$ in AT_2-null mice. These observations suggest that the AT_2 receptor has a tonic inhibitory action on the production of PGI_2 and PGE_2, which is driven by the AT_1 receptor.

It is generally appreciated that Ang II can stimulate PGI_2 and PGE_2 production in nonrenal tissues. Studies

using isolated vascular smooth muscle cells and isolated perfused heart preparations have demonstrated that Ang II stimulates PGI_2 release through an interaction at AT_1 receptors (11–15). However, the angiotensin subtype receptor responsible for renal PGE_2 and PGI_2 formation has not been studied systematically. The results of the present study show that both renal PGI_2 and PGE_2 production are stimulated in parallel by either endogenous (sodium restriction) or exogenous Ang II.

PGI_2 and PGE_2 are both formed from the precursor PGH_2, which is a product of cyclooxygenase action on its substrate, arachidonic acid. $PGF_{2\alpha}$, another major renal prostanoid, can also be formed directly from PGH_2 or, alternatively, by conversion from PGE_2 through the action of PGE_2 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). PGE_2 and $PGF_{2\alpha}$ 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 A_2, 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 PGE_2 production through the AT_1 receptor (6). Our present results are consistent with earlier observations in the normal rat kidney that conversion of PGE_2 to $PGF_{2\alpha}$ by 9-ketoreductase is enhanced by sodium depletion (22, 23).

An important finding of the present study is that Ang II acts at the AT_2 receptor to stimulate $PGF_{2\alpha}$, probably by conversion from PGE_2. At baseline, mice lacking the AT_2 receptor had increased RIF levels of PGE_2 and low levels of $PGF_{2\alpha}$. In WT mice, both sodium restriction (endogenous Ang II) and chronic infusion of a small quantity of exogenous Ang II increased 6-keto-$PGF_{1\alpha}$, PGE_2, and $PGF_{2\alpha}$. Similarly, in AT_2-null mice, sodium restriction and chronic Ang II infusion increased 6-keto-$PGF_{1\alpha}$ and PGE_2. In marked contrast to WT mice, however, in AT_2-null mice, both exogenous and endogenous Ang II failed to increase $PGF_{2\alpha}$. These results were confirmed by experiments showing that sodium restriction increased PGE_2, PGI_2, and $PGF_{2\alpha}$, and by separate experiments showing that AT_2 receptor blockade with PD blocked the increase in PGE_2 in WT mice. Our results underscore the physiological importance of the AT_2 receptor in the formation of $PGF_{2\alpha}$, most likely through conversion from PGE_2 by 9-ketoreductase. Our data, however, do not permit determination of the effects, if any, of the AT_2 receptor on PGH_2 metabolism. Our data demonstrate that the increase in basal PGE_2

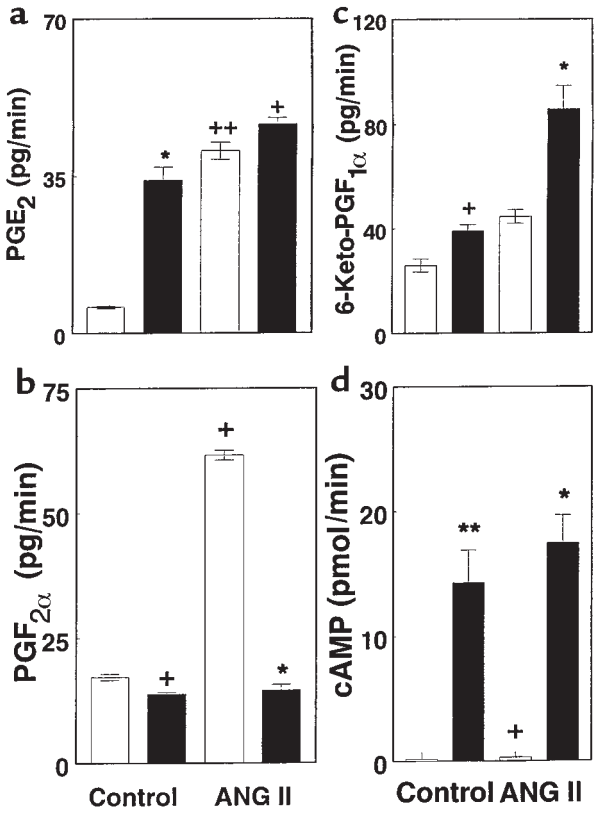

Figure 2
RIF PGE_2 (**a**), $PGF_{2\alpha}$ (**b**), 6-keto-$PGF_{1\alpha}$ (**c**), and cAMP (**d**) in mice (n = 10) lacking the AT_2 angiotensin receptor (filled bars) and in WT mice (n = 10; open bars) during the vehicle control period (Control) and after 7 days of continuous infusion of Ang II. (**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.

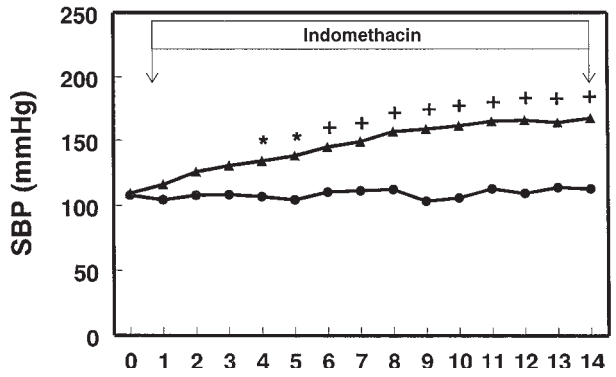

Figure 3
SBP of mice (n = 10) lacking the AT_2 angiotensin receptor (triangles) and of WT mice (n = 10, circles) during vehicle infusion (day 0) and during continuous intraperitoneal infusion of indomethacin (5 mg/kg/d) for 14 days. $^*P < 0.001$ vs. day 0 and WT. $^+P < 0.0001$ vs. day 0 and WT.

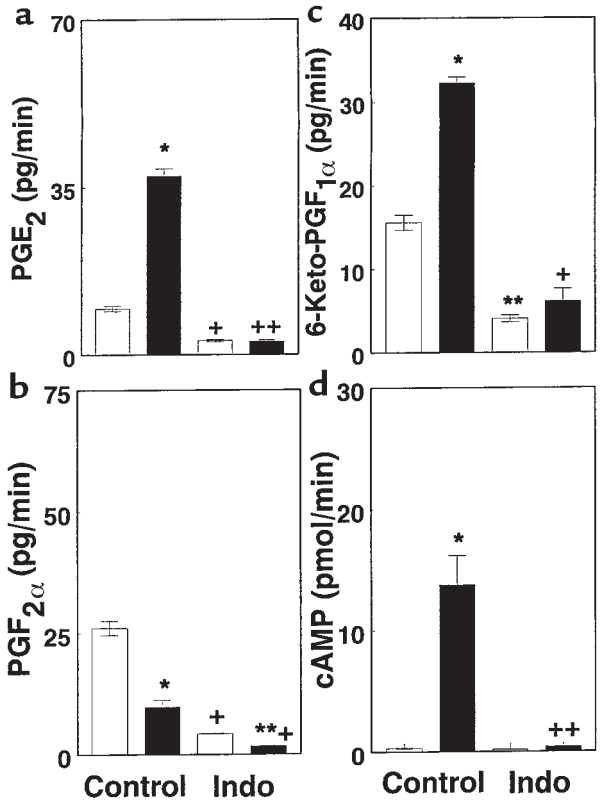

Figure 4

RIF PGE_2 (**a**), $PGF_{2\alpha}$ (**b**), 6-keto-$PGF_{1\alpha}$ (**c**), and cAMP (**d**) in mice (n = 10) lacking the AT_2 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 AT_2-null mice is largely related to absence of tonic inhibition of the AT_2 receptor on AT_1 receptor–mediated PGE_2 production.

The interaction between the renin-angiotensin system and renal eicosanoids is fundamental to the regulation of body fluid and sodium homeostasis (8). PGI_2 and PGE_2 stimulate, whereas $PGF_{2\alpha}$ inhibits, renin secretion (24). AT_2 receptor–mediated stimulation of $PGF_{2\alpha}$ formation, and its inhibitory effect on renin secretion, is in agreement with recent findings that the AT_2 receptor may indirectly mediate vasodilation (9). PGE_2 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 PGE_2 to $PGF_{2\alpha}$ is related to increased Ang II formation and stimulation of the AT_2 receptor. The decrease in sodium excretion during sodium restriction is likely mediated by AT_1 receptors in renal tubule cells in both WT and AT_2-null mice.

The tonic inhibitory action of the AT_2 receptor on AT_1 receptor–mediated PGI_2 and PGE_2 production may be due to several possible factors. (a) Nitric oxide, which is increased by AT_2 receptors, has been described to inhibit cyclooxygenase under some conditions (27, 28). (b) cGMP, also increased by AT_2 receptors, may affect prostaglandin metabolism (28). (c) Bradykinin, also increased by AT_2 receptors, can stimulate the production of nitric oxide and eicosanoids in the intact kidney (29). (d) The AT_2 receptor may stimulate epoxide production through the cytochrome P_{450} system, which may compete with arachidonic acid for metabolism by cyclooxygenase, resulting in inhibition of PGI_2 and PGE_2 formation (30, 31). These possibilities will need to be resolved in future studies.

$PGF_{2\alpha}$, the product of PGE_2 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. $PGF_{2\alpha}$ also can bind to EP_1 and EP_3 receptors, both of which also mediate vasoconstriction (32). In AT_2-null mice, the decrease in $PGF_{2\alpha}$ may contribute to the absence of hypertension by reducing peripheral and/or renal vasoconstriction.

PGE_2 has been shown to stimulate adenylyl cyclase via the EP_2 and EP_4 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 AT_2-null mice (PGE_2 and PGI_2 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 PGE_2. The parallel reduction of PGE_2, 6-keto-$PGF_{1\alpha}$, and cAMP by indomethacin suggests, however, that PGE_2 and/or PGI_2 are very likely responsible for elevated cAMP in AT_2-null mice. Failure of cAMP to increase substantially in response to sodium restriction or Ang II in AT_2-null mice may indicate maximum stimulation of cAMP production in the basal state.

Both PGE_2 and PGI_2 are vasodilators, and excessive vasodilator prostanoid accumulation in the absence of the AT_2 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-$PGF_{1\alpha}$, PGE_2, and $PGF_{2\alpha}$ to low levels, BP steadily rose into the hypertensive range. These results suggest that increased PGI_2 and PGE_2 in AT_2-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 AT_2 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

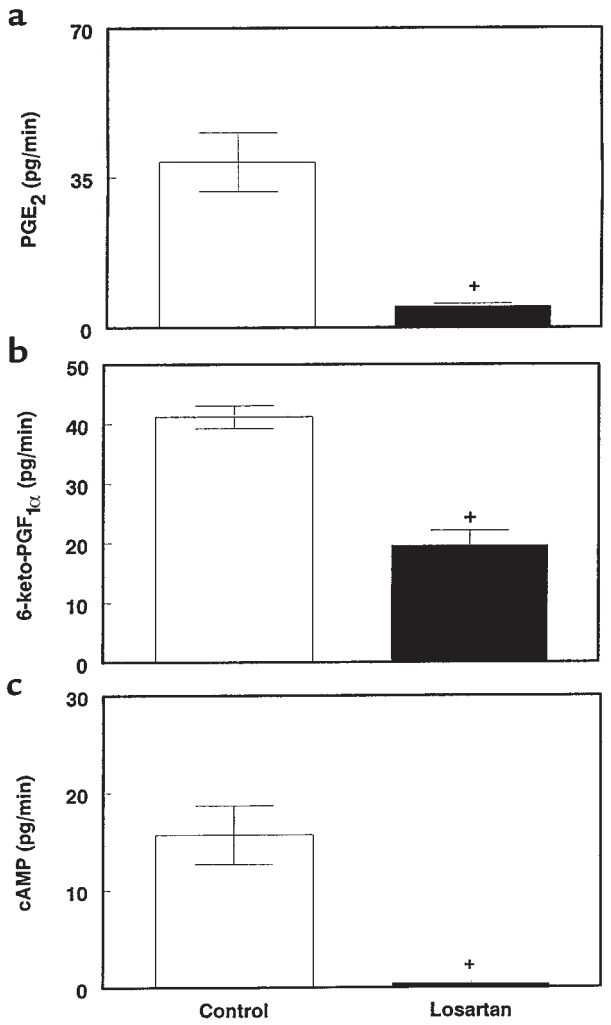

Figure 5
RIF PGE_2 (**a**), 6-keto-$PGF_{1\alpha}$ (**b**), and cAMP (**c**) in WT mice (n = 5) before and 2 hours after intravenous losartan administration (10 mg/kg). $^{+}P < 0.0001$ vs. control.

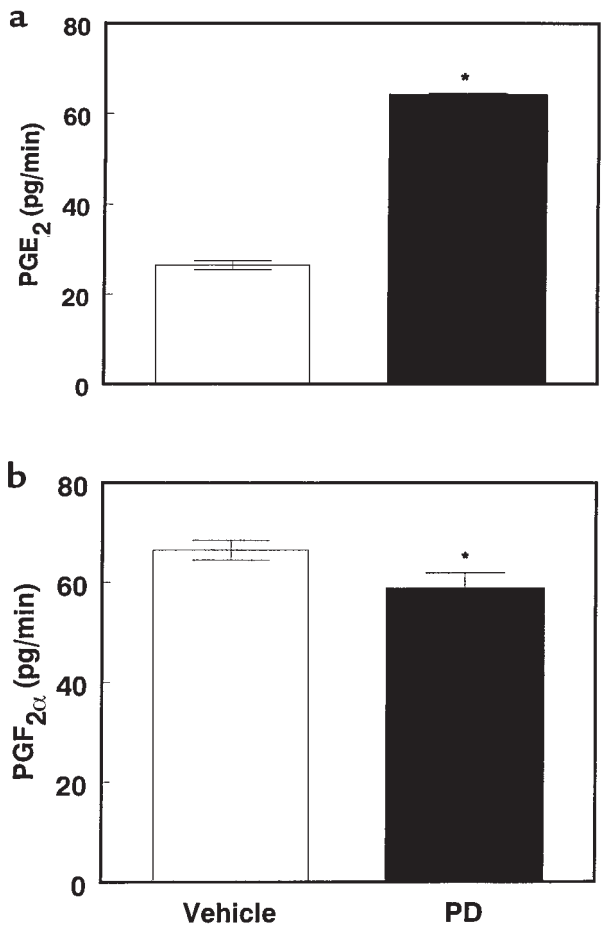

Figure 6
Incremental increase in RIF PGE_2 (**a**) and $PGF_{2\alpha}$ (**b**) in WT mice (n = 5) in response to 5 days of dietary sodium restriction in the presence or absence of the AT_2 receptor antagonist PD123319 (PD; 50 μg/kg/min). $^{*}P < 0.001$ vs. vehicle control.

altered RIF autacoid levels. (b) Although we were able to monitor PGE_2 and $PGF_{2\alpha}$ levels, we were not able to measure the activity of PGE_2 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 Ca^{2+} 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 AT_2 receptor results in increased PGI_2 and PGE_2 and decreased $PGF_{2\alpha}$ levels in the kidney, both basally and in response to Ang II. These findings establish a physiological role of the AT_2 receptor in renal prostanoid production and metabolism. We also demonstrated that the renal vasodilator prostanoids PGE_2 and PGI_2 are stimulated via the AT_1 receptor, providing counterregulatory vasodilation in opposition to the vasoconstrictor action of Ang II. Most importantly, we demonstrated that in the absence of the AT_2 receptor, increased levels of these vasodilator prostanoids prevent the development of hypertension.

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