Angiotensin II-induced Hypertension in the Rat

EFFECTS ON THE PLASMA CONCENTRATION, RENAL EXCRETION, AND TISSUE RELEASE OF PROSTAGLANDINS

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ABSTRACT We examined in rats the effects of intraperitoneal angiotensin II (AII) infusion for 12 d on urinary excretion, plasma concentration, and in vitro release of prostaglandin (PG) E_2 and 6-keto-$PGF_{1\alpha}$, a PGI_2 metabolite. AII at 200 ng/min increased systolic blood pressure (SBP) progressively from 125±3 to 170±9 mmHg ($P < 0.01$) and elevated fluid intake and urine volume. Urinary 6-keto-$PGF_{1\alpha}$ excretion increased from 38±6 to 55±5 and 51±7 ng/d ($P < 0.05$) on days 8 and 11, respectively, of AII infusion, but urinary PGE_2 excretion did not change. Relative to a control value of 129±12 pg/ml in vehicle-infused (V) rats, arterial plasma 6-keto-$PGF_{1\alpha}$ concentration increased by 133% ($P < 0.01$) with AII infusion. Aortic rings from AII-infused rats released more 6-keto-$PGF_{1\alpha}$ (68±7 ng/mg) during 15-min incubation in Krebs solution than did rings from V rats (40±3 ng/mg); release of PGE_2, which was <1% of that of 6-keto-$PGF_{1\alpha}$, was also increased. Slices of inner renal medulla from AII-infused rats released more 6-keto-$PGF_{1\alpha}$ (14±1 ng/mg) during incubation than did slices from V rats (8±1 ng/mg, $P < 0.05$), but PGE_2 release was not altered. In contrast, AII infusion did not alter release of 6-keto-$PGF_{1\alpha}$ or PGE_2 from inferior vena cava segments or from renal cortex slices. Infusion of AII at 125 ng/min also increased SBP, plasma 6-keto-$PGF_{1\alpha}$ concentration, and in vitro release of 6-keto-$PGF_{1\alpha}$ from rings of aorta and renal inner medulla slices; at 75 ng/min AII had no effect. SBP on AII infusion day 11 correlated positively with both 6-keto-$PGF_{1\alpha}$ plasma concentration ($r = 0.54$) and net aortic ring release ($r = 0.70$) when data from all rats were combined. We conclude that augmentation of PGI_2 production is a feature of AII-induced hypertension. The enhancement of PGI_2 production may be an expression of nonspecific alteration in vascular structure and metabolic functions during AII-induced hypertension, as well as the result of a specific effect of the peptide on the arachidonate-prostaglandin system.

INTRODUCTION

The concept that an interplay between prostaglandins (PG)[1] and angiotensin II contributes to circulatory homeostasis was first advanced in 1970 by McGiff et al. (1), who reported release of a PG-like substance into renal venous blood in response to arterial angiotensin II infusion. Subsequently, this notion gained strength with reports that the vascular effects of angiotensin II are augmented by prostaglandin synthetase inhibitors (2–4) and attenuated by both PGE_2 and PGI_2 (5, 6) and that, in conditions featuring activation of the renin-angiotensin system, the administration of prostaglandin synthetase inhibitors causes renal vasoconstriction that is correlated positively with the level of plasma renin activity (7). It is now also established that angiotensin II can promote release of PG from a variety of organs, including kidneys (1, 2, 8), lungs (9, 10), heart (3, 11), and blood vessels (12, 13), and that this action is due to stimulation of PG biosynthesis (11). However, much of the information on the effects of angiotensin II on PG biosynthesis is derived from short-term studies of isolated perfused organs or of anesthetized animals subjected to surgical stress and may not, consequently, be applicable to more physiological settings. For example, it is conceivable that the PG

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[1] *Abbreviations used in this paper:* PG, prostaglandin(s).

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response of a tissue or organ to a brief and localized increase in angiotensin II varies from the response to a prolonged and systemic increase; in the latter case the response may vary with time, and be affected by the many functional and metabolic alterations that are associated with chronic increases in angiotensin II levels. If so, any further consideration of an angiotensin II-PG interplay in long-term regulation of physiological events requires information on the consequences of prolonged elevation of angiotensin II levels on the PG system. The present study in conscious rats was, therefore, designed to assess the effects of chronic angiotensin II administration on the urinary excretion, plasma concentration, and in vitro tissue release of PGE_2 and 6-keto-$PGF_{1\alpha}$; the latter arises from nonenzymatic hydrolysis of PGI_2 (11), and is viewed as an expression of PGI_2 levels and production.

METHODS

Experiments were performed on 51 male Wistar rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 231–290 g; 27 of the rats were housed in individual metabolism cages, and the remainder were maintained in group cages with three to five rats per cage. The rats were kept in a temperature (24°C)- and humidity (50%)-controlled room that was illuminated between 6:00 a.m. and 6:00 p.m., had free access to tap water, and were fed ad lib. a standard chow (Purina No. 5001, Ralston Purina Co., St. Louis, MO) containing sodium (174 meq/kg) and potassium (282 meq/kg).

Protocol. Studies were performed after a 7-d period of acclimatization of the rats to the housing, feeding, and drinking conditions. Following a subsequent 4-d control period, each animal was anesthetized with ether and an Alzet osmotic minipump (model 2002, Alza Corp., Palo Alto, CA) filled with angiotensin II solution or vehicle was placed in the abdominal cavity through a 1-cm midline incision, which was then closed with autoclips. Isoleucine^5-angiotensin II (Sigma Chemical Co., St. Louis, MO) was dissolved to different concentrations in 0.01 N acetic acid. Rats serving as time controls received only 0.01 N acetic acid. Assuming that the angiotensin II did not degrade during the study, and that the pumps dispensed fluid at the specified rate of 0.49 μl/h, the calculated infusion rates of angiotensin II were 75, 125, and 200 ng/min, in 9, 4, and 19 rats, respectively. Aguilera et al. (14) reported that intraperitoneal angiotensin II infusion at doses ranging from 50 to 250 ng/min elevated the concentration of angiotensin II in blood by about three- to 40-fold after 36 h of infusion.

Systolic blood pressure and body weight were determined in all rats during the control period and at intervals after implantation of the minipumps: in rats housed in individual metabolism cages, 24-h food and electrolyte consumption, water intake, urine volume, and urinary excretion of sodium, potassium, PGE_2, and 6-keto-$PGF_{1\alpha}$ were also determined. Urine was collected for 24-h periods at room temperature except during measurement of PG excretion, in which case the urine was received in plastic bottles surrounded by dry ice to keep the specimens frozen throughout the collection period.

On day 4 or 12 of the infusion period, the rats were anesthetized with ether and the abdomen was opened with a midline incision. The abdominal aorta was punctured and a 4–6-ml blood sample was collected into a syringe containing 15% EDTA (100 μl) and indomethacin (100 μg in 10 μl ethanol): after centrifugation the plasma was frozen for later determination of PG concentration. The thoracic aorta, the supradiaphragmatic inferior vena cava, and the left kidney were then excised in some of the rats for estimation of in vitro release of PG.

PG release studies. The aorta, vena cava, and kidney were placed in ice-cold Krebs solution containing (mM) NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.17, dextrose 8.4, and $NaHCO_3$ 25.0, and rinsed free of visible blood. The thoracic aorta was freed of adventitial tissue and cut into 2-mm rings; the inferior vena cava was cut into 4–5-mm segments. The kidney was decapsulated and bisected longitudinally, the inner medulla was excised and segmented into slices with a blade, and the cortex was sliced (0.5 mm) with a Stadie-Riggs microtome. The vascular and renal tissues were placed into 25-ml flasks containing 2 ml of Krebs solution and were incubated for 15 min at 37°C under an atmosphere of 95% O_2–5% CO_2, with 100 cycle/min agitation. At the termination of the incubation the medium was frozen for later PG radioimmunoassay. Tissue specimens in each incubation flask were dried to constant weight, averaging 1.31 mg for the aorta, 2.68 mg for the vena cava, 13.27 mg for the renal cortex, and 3.06 mg for the renal inner medulla. Results are expressed as nanograms of immunoreactive PG released during the 15-min incubation period per milligram of dry tissue.

Radioimmunoassay of PGE_2 and 6-keto-$PGF_{1\alpha}$. Test samples and radioimmunoassay reagents were diluted with 0.1 M phosphate buffer, pH 7.4, containing 0.15 M sodium chloride, 0.1% sodium azide, and 0.1% gelatin. Reaction mixtures consisting of equal volumes (100 μl) of tritium-labeled PGE_2 or 6-keto-$PGF_{1\alpha}$ (8,000 dpm; New England Nuclear, Boston, MA), an appropriate antibody diluted to bind 40% of the tracer in the absence of unlabeled ligand, and the unknown sample or the corresponding PG standard (Upjohn Co., Kalamazoo, MI) were incubated at 4°C for 16 h; after addition of dextran-coated charcoal the suspension was centrifuged and the radioactivity of the supernate was determined. The PGE_2 antibody (Institute Pasteur) had <0.5% cross-reactivity with $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGD_2, and PGA_2. The 6-keto-$PGF_{1\alpha}$ antibody was a gift from Dr. Lawrence Levine (Department of Biochemistry, Brandeis University, Waltham, MA) and cross-reacted <0.5% with PGE_2, $PGF_{2\alpha}$, PGD_2, and 6-keto-$PGE_{1\alpha}$. The amount of PG standard required to produce 50% displacement of radiolabeled PG from antibody-binding sites was 14 pg for PGE_2, and 56 pg for 6-keto-$PGF_{1\alpha}$. PGE_2 and 6-keto-$PGF_{1\alpha}$ in the incubation media from the PG release studies were measured in unextracted samples diluted with the radioimmunoassay buffer. Alkali treatment (pH 12 at 100°C for 5 min) of incubation media samples did not affect the estimates of 6-keto-$PGF_{1\alpha}$, but resulted in complete disappearance of the PGE_2 immunoreactive material. The PG content of the urine and plasma was determined after extraction. The displacement of the tritium-labeled PGE_2 and 6-keto-$PGF_{1\alpha}$ from their corresponding antibodies by serially diluted samples of incubation media or of lipids extracted from plasma or urine paralleled the displacement caused by varying amounts of the appropriate PG standard.

To extract PG from urine the specimen (2 ml) was acidified to pH 3.0 with formic acid and passed through a column of octadecylsilyl silica (Sep Pak C_{18} cartridges, Waters Associates, Milipore Corp., Milford, MA) prewashed with methanol (2 ml) and then with water (20 ml); the column was then eluted successively with water (20 ml), ethanol/

water (15:85, vol/vol, 20 ml), petroleum ether (20 ml), and methyl formate (10 ml) (15). The methyl formate fraction containing the prostanoids was evaporated to dryness under a stream of nitrogen and the dry residue was reconstituted in toluene/ethyl acetate/methanol (60:40:1, vol/vol) and applied to a silica column (Sep Pak silica cartridges, Waters Associates) that had been prewashed with 2 ml of toluene/ethyl acetate/methanol/formic acid (60:40:20:1, vol/vol) and equilibrated with 20 ml of toluene/ethyl acetate (60:40, vol/vol). The column was eluted first with 10 ml of toluene/ethyl acetate (60:40, vol/vol) and then with 12 ml of toluene/ethyl acetate/methanol/formic acid (60:40:5:1, vol/vol); the latter fraction, containing the prostanoids, was dried under a stream of nitrogen and was reconstituted in the radioimmunoassay buffer. The recovery of 6-keto-[^{3}H]$PGF_{1\alpha}$ added to each urine sample before purification was 85.4±2.6% (n = 108): the individual 6-keto-$PGF_{1\alpha}$ recovery values served to correct the estimates of both 6-keto-$PGF_{1\alpha}$ and PGE_2 for losses incurred during purification, as in preliminary studies the recoveries of these PG added separately to urine did not differ from each other. The urinary excretion of PGE_2 and of 6-keto-$PGF_{1\alpha}$ was calculated as the product of 24-h urine volume and urinary PG concentration, and is expressed as nanograms per day. The coefficient of variation of PG assays in the same urine sample on seven different days was 12.9% for PGE_2 and 8.2% for 6-keto-$PGF_{1\alpha}$.

To extract PG from plasma the specimen (2–3 ml) was acidified to pH 3.0 with formic acid and applied to an octadecylsilyl silica column that was eluted as described above for urine samples. The methyl formate fraction containing the prostanoids was evaporated to dryness under nitrogen and the lipid residue was dissolved in methanol, applied as a band to a 0.25-mm thick silica gel G thin-layer chromatography plate (Redi-Plate, 20 × 20 cm, Fisher Scientific Co., Pittsburgh, PA), and chromatographed concurrently with authentic prostanoid standards, using as a solvent system the organic phase of ethyl acetate/isooctane/acetic acid/water (11:5:2:10, vol/vol). Authentic PG were located by exposing the plate to iodine vapor. Zones of the plate corresponding to the position of PGE_2 and 6-keto-$PGF_{1\alpha}$ were scraped off and eluted with methanol. The eluates were dried in nitrogen and the lipid residues were reconstituted in radioimmunoassay buffer and assayed for PGE_2 and 6-keto-$PGF_{1\alpha}$, respectively. The recovery of [^{3}H]PGE_2 and 6-keto-[^{3}H]$PGF_{1\alpha}$ added to each plasma sample before purification was 68.3±2.5 and 55.9±3.1%, respectively. The individual recovery values served to correct the estimates of plasma PGE_2 and 6-keto-$PGF_{1\alpha}$ for losses incurred during purification. The plasma levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ are expressed as picograms per milliliter. The coefficient of variation of PG assays on 7 different days in the same sample of plasma was 14.2% for PGE_2 and 13.0% for 6-keto-$PGF_{1\alpha}$.

Radioimmunoassay of 13,14-dihydro-15-keto-PGE_2. The concentration of this metabolite of PGE_2 in plasma was measured in unextracted specimens with an antiserum donated by Dr. Lawrence Levine (16); the plasma levels are expressed in picograms per milliliter. The intraassay coefficient of variation was 7.6%.

Systolic blood pressure determination. Systolic blood pressure was determined by tail sphygmography after warming the rats at 37°C for 10–15 min.

Urinary electrolytes and osmolality determinations. Sodium and potassium concentration in urine was determined by flame photometry with lithium as the internal standard, and urine osmolality was determined by freezing point depression.

Statistical analyses. Results are expressed throughout the text, tables and figures as mean±SEM. Data were analyzed initially by one- or two-way analysis of variance, followed by unpaired Student's t test or Newman-Keul's a posteriori test to evaluate differences. The null hypothesis was rejected when the P value was <0.05.

RESULTS

The effect of intraperitoneal infusion of angiotensin II on systolic blood pressure and body weight is shown in Fig. 1. Blood pressure increased progressively with the administration of angiotensin II at 125 or 200 ng/min, reaching values ~50 mmHg above the preinfusion level by the 11th d; blood pressure also increased on the 2nd d of infusion of 75 ng/min, but fell on subsequent days to values not different from those in vehicle-infused rats. Body weight gain was not affected by administration of angiotensin II for 11 d at 75 ng/min (+50±2 g), but it was reduced in rats receiving 125 ng/min (+13±5 g, $P < 0.01$) and arrested in rats receiving 200 ng/min (−12±8 g, $P < 0.01$), relative to values in vehicle-infused rats (+55±4 g). Failure of the rats infused with angiotensin II at 200 ng/min to grow was associated with a reduction in the 11-d cumulative food intake (171±11 g, $P < 0.01$) compared with food intake by the vehicle-infused rats (244±5 g); infusion of angiotensin II at 75 ng/d did not affect cumulative food intake (244±6 g).

Shown in Table I are fluid intake and urine volume, osmolality, and electrolyte values. Coincident with the increase in blood pressure, animals receiving angiotensin II at 200 ng/min exhibited a two- to threefold elevation in urine volume that was associated with a substantial increase of water intake, and a lowering of urinary osmolality to about one-third of the preinfusion value. The daily dietary intake of sodium and potassium was reduced throughout the period of administration of angiotensin II at 200 ng/min, and the urinary excretion of these electrolytes was either unchanged or decreased relative to values in vehicle-infused rats. However, for both sodium and potassium the difference between dietary intake and urinary excretion tended to fall in the rats infused with angiotensin II at 200 ng/min, suggesting reduced retention. Infusion of angiotensin II at 75 ng/min had no effect on any of the above discussed variables.

Fig. 2 depicts the urinary excretion of immunoreactive PGE_2 and 6-keto-$PGF_{1\alpha}$ before and during administration of angiotensin II. Relative to values in vehicle-infused rats, urinary 6-keto-$PGF_{1\alpha}$ was significantly increased on days 8 and 11 of angiotensin II infusion at 200 ng/min. In contrast, the administration of angiotensin II at this rate did not change the urinary output of PGE_2; at an infusion rate of 75 ng/min, angiotensin II did not affect the rates of urinary excretion of either PGE_2 or 6-keto-$PGF_{1\alpha}$.

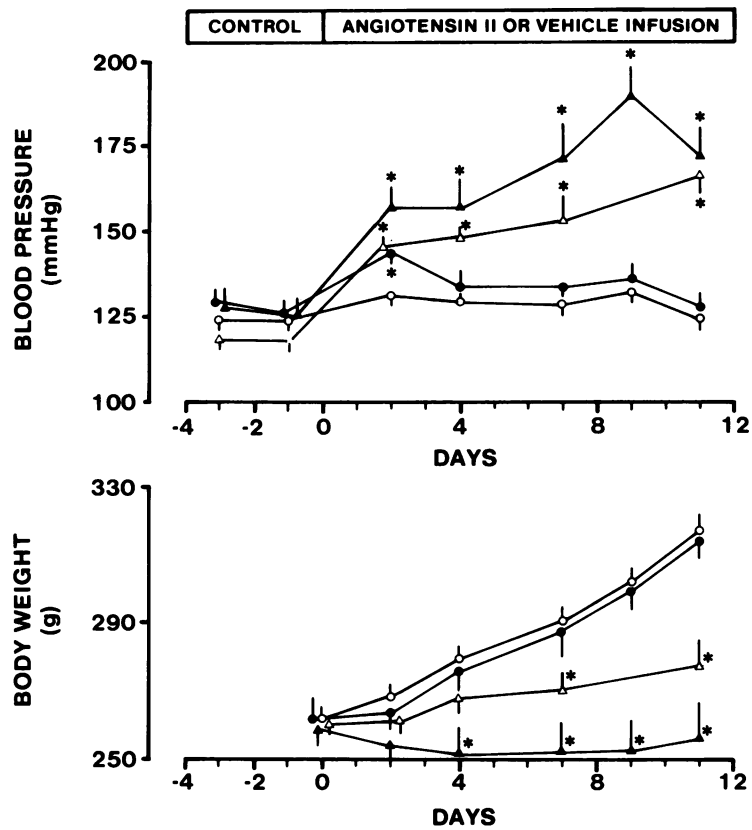

FIGURE 1 Systolic blood pressure and body weight before and during intraperitoneal infusion of angiotensin II (AII) in rats. Results are expressed as means±SE, n = number of rats. Asterisks indicate $P < 0.05$ relative to corresponding values in vehicle-infused rats. ○: vehicle, n = 14; ●: AII 75 ng/min, n = 9; △: AII 125 ng/min, n = 4; ▲: AII 200 ng/min, n = 14.

Plasma PG levels measured in arterial blood on the 12th d of infusion are displayed in Fig. 3. The level of immunoreactive 6-keto-$PGF_{1\alpha}$ increased by ~55% ($P < 0.05$) and 133% ($P < 0.01$) with the administration of angiotensin II for 12 d at 125 and 200 ng/min, respectively, relative to a control value of 129±12 pg/ml in vehicle-infused rats. The plasma level of 6-keto-$PGF_{1\alpha}$ also was higher ($P = 0.05$) in rats receiving angiotensin II for 4 d at 200 ng/min (152±19 pg/ml; $n = 5$) than in animals receiving vehicle only (98±14 pg/ml; $n = 5$). Plasma PGE_2 concentration was not affected by angiotensin II infused at 75 or 125 ng/min for 12 d, but tended to increase the rats infused at 200 ng/min ($0.01 < P < 0.1$). The plasma concentration of 13,14-dihydro-15-keto-PGE_2 in rats infused with angiotensin II for 12 d at 200 ng/min, but not at 75 ng/min, was higher ($P < 0.05$) than the concentration in animals receiving vehicle only. Linear regression analysis of combined data from the vehicle-infused rats and all three groups of rats infused with angiotensin II for 12 d revealed a positive correlation between plasma 6-keto-$PGF_{1\alpha}$ concentration and systolic blood pressure ($r = 0.54$; $P < 0.01$; $y = -91.7 + 1.92x$).

Net release of PG from vascular tissues during incubation in Krebs solution for 15 min is illustrated in Fig. 4. Release of PGE_2 from the aorta and the vena cava represented <0.4 and 4.2% of the net release of 6-keto-$PGF_{1\alpha}$, respectively. The net release of PG by vena cava segments taken from animals infused with angiotensin II for 12 d did not differ significantly from the release by vena cava segments taken from vehicle-infused controls. In contrast, rings of aorta from rats infused with angiotensin II for 12 d at 125 or 200 ng/min released more 6-keto-$PGF_{1\alpha}$ and PGE_2 than did rings from vehicle-infused rats. However, the release of 6-keto-$PGF_{1\alpha}$ by rings of aorta from rats receiving angiotensin II at 200 ng/min for 4 d only (49.4±8.4 ng/mg; $n = 5$) was not statistically different from the release by rings taken from animals injected with vehicle (45.9±6.1 ng/mg; $n = 5$); also the release of PGE_2 was not different. When data from all the animals infused with angiotensin II or vehicle for 12 d were com-

TABLE I

Fluid Intake, Urine Volume and Osmolality, Intake and Urinary Output of Electrolytes in Rats Infused with Angiotensin II or Vehicle

	Control			Infusion of angiotensin II (AII) or vehicle (V)										
Days	−2	−1	0	1	2	3	4	5	6	7	8	9	10	11
Water intake (*ml/24 h*)														
V	35.5	40.0	34.2	29.5	38.3	32.8	39.0	38.8	37.5	38.9	36.4	37.2	40.1	38.4
	±1.5	±1.6	±1.5	±1.6	±1.5	±1.2	±1.6	±1.0	±1.8	±1.0	±1.9	±1.3	±2.2	±2.0
AII 75	33.3	36.1	34.4	26.7	39.6	38.6	40.1	40.8	39.3	40.0	38.7	38.6	44.7	35.0
(*ng/min*)	±0.9	±1.5	±1.7	±2.4	±1.8	±2.7	±2.6	±3.8	±1.5	2.6	±2.6	±2.6	±2.8	±1.4
AII 200	34.0	38.0	33.7	25.7	42.4	50.0*	53.4*	54.6*	58.3*	54.2*	54.6*	54.0*	60.4*	54.0*
(*ng/min*)	±1.2	±0.9	±1.2	±2.0	±4.5	±6.9	±7.4	±7.4	±7.6	±5.8	±6.0	±7.0	±7.0	±6.3
Urine volume (*ml/24 h*)														
V	11.6	13.6	11.5	7.6	11.7	10.4	13.0	10.4	12.2	9.7	13.0	10.7	12.6	12.3
	±1.0	±1.2	±0.7	±0.4	±0.8	±0.8	±0.9	±0.4	±0.6	±0.3	±0.7	±0.5	±0.6	±1.1
AII 75	10.2	12.9	12.4	9.7	15.3	12.3	16.9	12.9	13.7	11.6	15.8	13.6	15.7	13.9
(*ng/min*)	±0.6	±1.0	±1.1	±1.2	±3.2	±1.8	±2.8	±1.4	±2.1	±1.8	±2.1	±3.0	±1.9	±1.7
AII 200	10.5	12.9	12.5	16.0*	28.2*	29.8*	32.8*	34.5*	36.3*	31.8*	37.0*	35.0*	35.0*	36.0*
(*ng/min*)	±0.6	±1.2	±1.2	±1.7	±3.6	±6.5	±7.5	±6.8	±7.8	±5.6	±5.5	±7.2	±7.2	±7.3
Urine osmolality (*mosmol/kg H_2O*)														
V	1,700						1,684				1,717			1,639
	±88						±68				±99			±79
AII 75	1,680						1,485				1,494			1,577
(*ng/min*)	±98						±129				±122			±79
AII 200	1,612						686*				604*			548*
(*ng/min*)	±77						±83				±85			±81
Sodium intake (*meq/24 h*)														
V	4.01	4.13	3.93	2.47	3.96	3.74	3.90	4.06	4.16	3.86	4.02	3.71	4.26	4.15
	±0.08	±0.07	±0.10	±0.28	±0.10	±0.13	±0.28	±0.09	±0.16	±0.04	±0.16	±0.12	±0.14	±0.18
AII 75	3.91	3.98	4.05	2.52	3.52	3.82	4.04	4.07	4.22	3.79	4.10	3.81	4.27	4.17
(*ng/min*)	±0.14	±0.09	±0.13	±0.18	±0.20	±0.15	±0.17	±0.17	±0.09	±0.15	±0.11	±0.10	±0.13	±0.15
AII 200	3.90	4.16	3.85	1.98	2.10*	2.55*	2.82*	2.94*	3.41*	2.84*	2.85*	2.46*	3.04*	3.00*
(*ng/min*)	±0.10	±0.10	±0.10	±0.35	±0.23	±0.18	±0.17	±0.27	±0.26	±0.21	±0.39	±0.17	±0.26	±0.40

Sodium excretion (*meq/24 h*)

V	2.60	2.74	2.56	1.58	2.60	2.40	2.62	2.32	2.76	2.29	2.54	2.51	2.75	2.34
	±0.15	±0.10	±0.06	±0.10	±0.13	±0.17	±0.17	±0.17	±0.14	±0.15	±0.15	±0.17	±0.18	±0.22
AII 75	2.56	2.80	2.90	1.64	2.58	2.10	3.04	2.45	3.05	2.31	2.58	2.56	2.88	2.54
(*ng/min*)	±0.07	±0.13	±0.21	±0.11	±0.25	±0.19	±0.18	±0.12	±0.17	±0.15	±0.17	±0.14	±0.15	±0.26
AII 200	2.49	2.61	2.72	1.70	2.11	1.50*	2.25	2.37	2.53	2.33	2.18	1.86*	2.10*	2.10
(*ng/min*)	±0.16	±0.11	±0.19	±0.11	±0.24	±0.21	±0.22	±0.24	±0.15	±0.26	±0.19	±0.25	±0.22	±0.17

Sodium intake-excretion difference (*meq/24 h*)

V	1.41	1.39	1.37	0.89	1.36	1.34	1.28	1.74	1.40	1.57	1.48	1.20	1.51	1.81
	±0.12	±0.05	±0.10	±0.29	±0.13	±0.16	±0.23	±0.15	±0.15	±0.15	±0.19	±0.15	±0.18	±0.23
AII 75	1.35	1.18	1.15	0.88	1.36	1.34	1.28	1.74	1.40	1.57	1.48	1.20	1.51	1.81
(*ng/min*)	±0.10	±0.10	±0.22	±0.10	±0.37	±0.24	±0.17	±0.23	±0.13	±0.15	±0.09	±0.14	±0.17	±0.23
AII 200	1.41	1.55	1.13	0.28	−0.01*	1.05	0.57*	0.57*	0.88	0.51*	0.67*	0.60	0.94	0.90*
(*ng/min*)	±0.14	±0.15	±0.20	±0.41	±0.28	±0.36	±0.23	±0.25	±0.25	±0.36	±0.27	±0.25	±0.33	±0.33

Potassium intake (*meq/24 h*)

V	6.50	6.71	6.39	4.02	6.00	6.08	6.33	6.59	6.76	6.24	6.53	6.02	6.92	6.74
	±0.13	±0.12	±0.16	±0.45	±0.27	±0.12	±0.46	±0.15	±0.26	±0.06	±0.26	±0.19	±0.23	±0.30
AII 75	6.35	6.47	6.59	4.09	5.73	6.21	6.57	6.61	6.89	6.15	6.83	6.18	6.93	6.77
(*ng/min*)	±0.22	±0.15	±0.21	±0.29	±0.32	±0.24	±0.22	±0.27	±0.15	±0.24	±0.22	±0.16	±0.21	±0.25
AII 200	6.34	6.76	6.26	3.21	3.45*	4.15*	4.58*	4.77*	5.53*	4.61*	4.62*	3.99*	4.93*	4.89*
(*ng/min*)	±0.15	±0.16	±0.16	±0.57	±0.36	±0.29	±0.27	±0.43	±0.42	±0.34	±0.35	±0.27	±0.42	±0.65

Potassium excretion (*meq-24 h*)

V	4.09	4.44	4.08	2.78	3.80	3.84	4.13	3.83	4.30	3.73	4.08	3.97	4.54	3.95
	±0.20	±0.15	±0.12	±0.08	±0.24	±0.28	±0.29	±0.16	±0.19	±0.19	±0.15	±0.22	±0.21	±0.33
AII 75	3.89	4.48	4.46	2.86	3.91	4.02	4.42	4.10	4.45	3.64	4.21	3.99	4.51	4.08
(*ng/min*)	±0.16	±0.14	±0.36	±0.21	±0.28	±0.37	±0.19	±0.10	±0.21	±0.17	±0.24	±0.25	±0.25	±0.46
AII 200	3.90	4.29	4.36	2.39*	2.95*	2.84*	2.90*	3.36	3.82	3.11*	3.23*	3.15*	3.50*	3.13
(*ng/min*)	±0.19	±0.10	±0.30	±0.13	±0.13	±0.11	±0.26	±0.23	±0.21	±0.18	±0.25	±0.27	±0.35	±0.37

Potassium intake-excretion difference (*meq/24 h*)

V	2.41	2.27	2.31	1.24	2.20	2.24	2.20	2.76	2.46	2.51	2.45	2.05	2.38	2.79
	±0.17	±0.08	±0.13	±0.43	±0.29	±0.27	±0.34	±0.17	±0.19	±0.18	±0.26	±0.21	±0.24	±0.34
AII 75	2.46	1.99	2.13	1.23	1.82	2.19	2.15	2.51	2.45	2.51	2.62	2.18	2.42	2.69
(*ng/min*)	±0.17	±0.13	±0.33	±0.17	±0.35	±0.41	±0.20	±0.24	±0.15	±0.25	±0.15	±0.27	±0.25	±0.39
AII 200	2.44	2.47	1.90	0.82	0.50*	1.31*	1.68	1.41*	1.71*	1.50*	1.39*	0.84*	1.43*	1.76
(*ng/min*)	±0.15	±0.17	±0.31	±0.50	±0.25	±0.27	±0.29	±0.24	±0.24	±0.25	±0.36	±0.26	±0.26	±0.34

Values are means±SE of nine observations. Asterisk denotes significant difference from corresponding value in vehicle-infused rats ($P < 0.05$; two-way analysis of variance and Newman-Keul test.

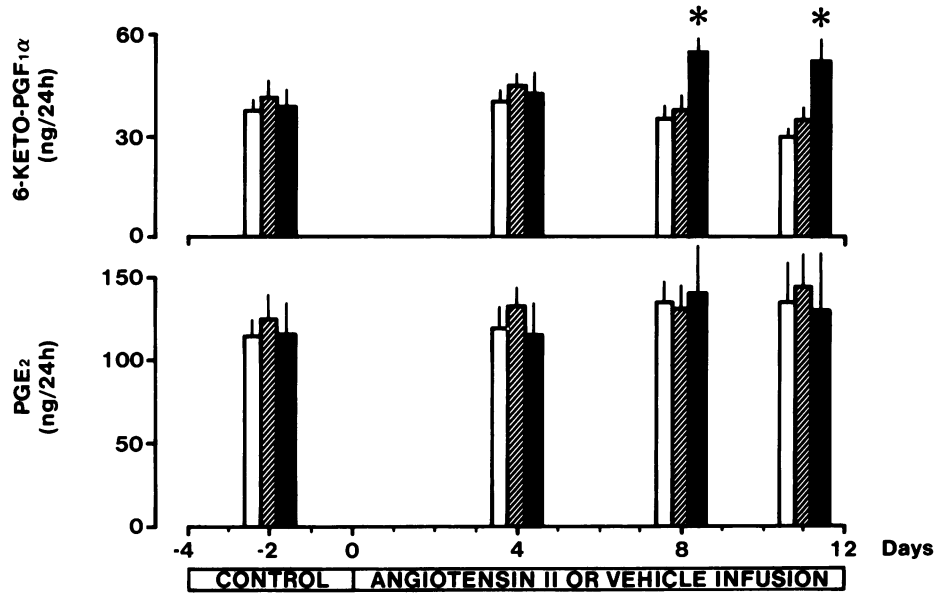

FIGURE 2 Urinary excretion of immunoreactive 6-keto-$PGF_{1\alpha}$ and PGE_2 before and during intraperitoneal infusion of angiotensin II (AII) in rats. Results are expressed as means±SE, n = number of rats. Asterisks indicate $P < 0.05$ relative to corresponding values in vehicle-infused rats. □: vehicle, n = 9; ▨: AII 75 ng/min, n = 9; ■: AII 200 ng/min, n = 9.

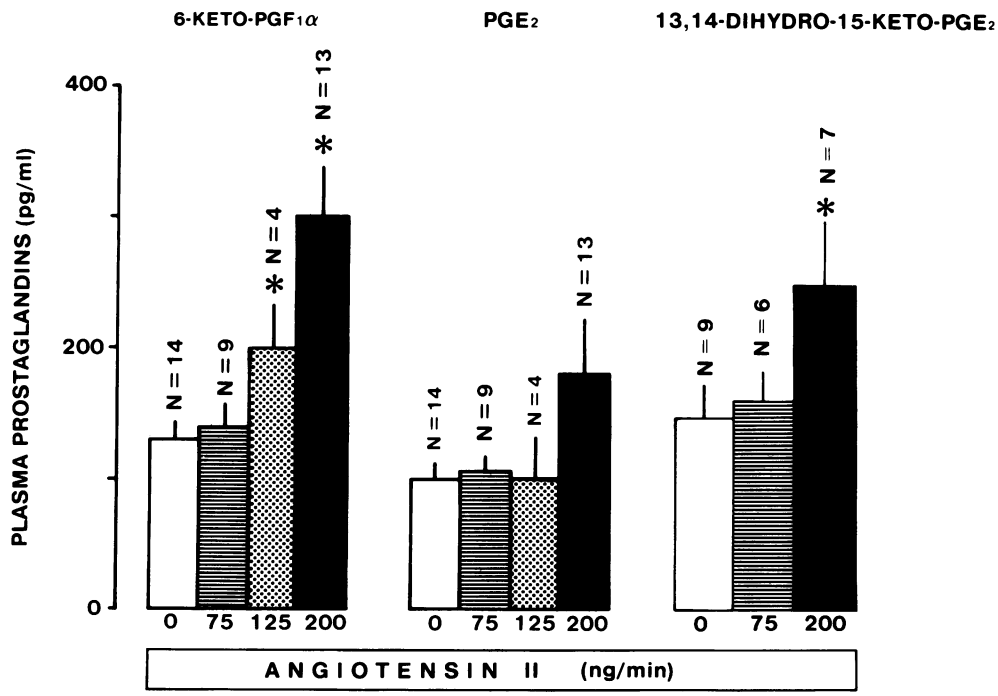

FIGURE 3 Immunoreactive 6-keto-$PGF_{1\alpha}$, PGE_2, and 13,14-dihydro-15-keto-PGE_2 concentrations in arterial plasma on the 12th d of angiotensin II intraperitoneal infusion. Results are expressed as means±SE, n = number of rats. Asterisks indicate $P < 0.05$ relative to values in vehicle-infused rats (open columns).

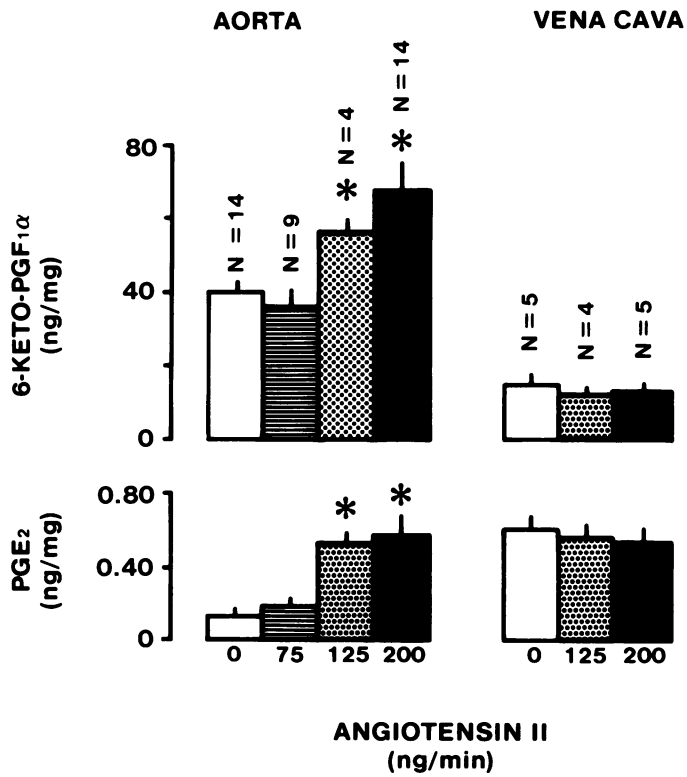

FIGURE 4 Net release of immunoreactive 6-keto-$PGF_{1\alpha}$ and PGE_2 from segments of thoracic aorta and inferior vena cava during incubation in Krebs solution for 15 min at 37°C. Results are expressed as means±SE, n = number of rats. Asterisks indicate $P < 0.05$ relative to values in vehicle-infused rats (open columns).

bined, as shown in Fig. 5, the net release of 6-keto-$PGF_{1\alpha}$ from rings of aorta correlated positively with blood pressure; PGE_2 release and blood pressure were similarly correlated. However, the release of PG from rings of aorta did not correlate significantly with blood pressure in rats receiving angiotensin II at 200 ng/min, or vehicle, for 4 d only (for 6-keto-$PGF_{1\alpha}$ $r = 0.21$; for PGE_2 $r = 0.24$).

Shown in Table II is the net release of PG from renal tissues during incubation in Krebs solution for 15 min. Slices of inner medulla from rats infused with angiotensin II at 125 or 200 ng/min for 12 d released more 6-keto-$PGF_{1\alpha}$ than did slices from vehicle-infused rats. In contrast, the net release of PGE_2 from inner medulla slices was not affected. Similarly, neither PGE_2 nor 6-keto-$PGF_{1\alpha}$ release from renal cortex slices obtained from angiotensin II-infused rats differed significantly from corresponding values for slices from vehicle-infused rats.

DISCUSSION

PGE_2 in the urine arises within the kidney (17) and its rate of excretion is a function of renal PGE_2 synthesis (18). In the present study a chronic infusion of angiotensin II did not affect either urinary PGE_2 excretion or net release of PGE_2 from renal tissue in vitro, suggesting that the renal production of PGE_2 did not increase. These findings appear to be at variance with reports that angiotensin II promotes renal PGE_2 synthesis and release. However, all such prior reports have been restricted to the effects on PG synthesis of short-term angiotensin II administration and the results, therefore, can not be considered to conflict directly with those of our present chronic infusion study. Further, data contained in two early reports (1, 8) of the effect of close renal arterial infusion of angiotensin II on renal PG show that renal PGE_2 release was augmented initially, but that the effect was not sustained. Rather, the angiotensin II infusion was shown to induce prompt rises in urinary PGE_2 excretion and renal venous blood PGE_2 concentration, and both tended to return to or toward control levels after several minutes of continued infusion.

Contrasting with the lack of effect of chronic angiotensin II infusion on urinary PGE_2 excretion, the urinary output of 6-keto-$PGF_{1\alpha}$ increased during an-

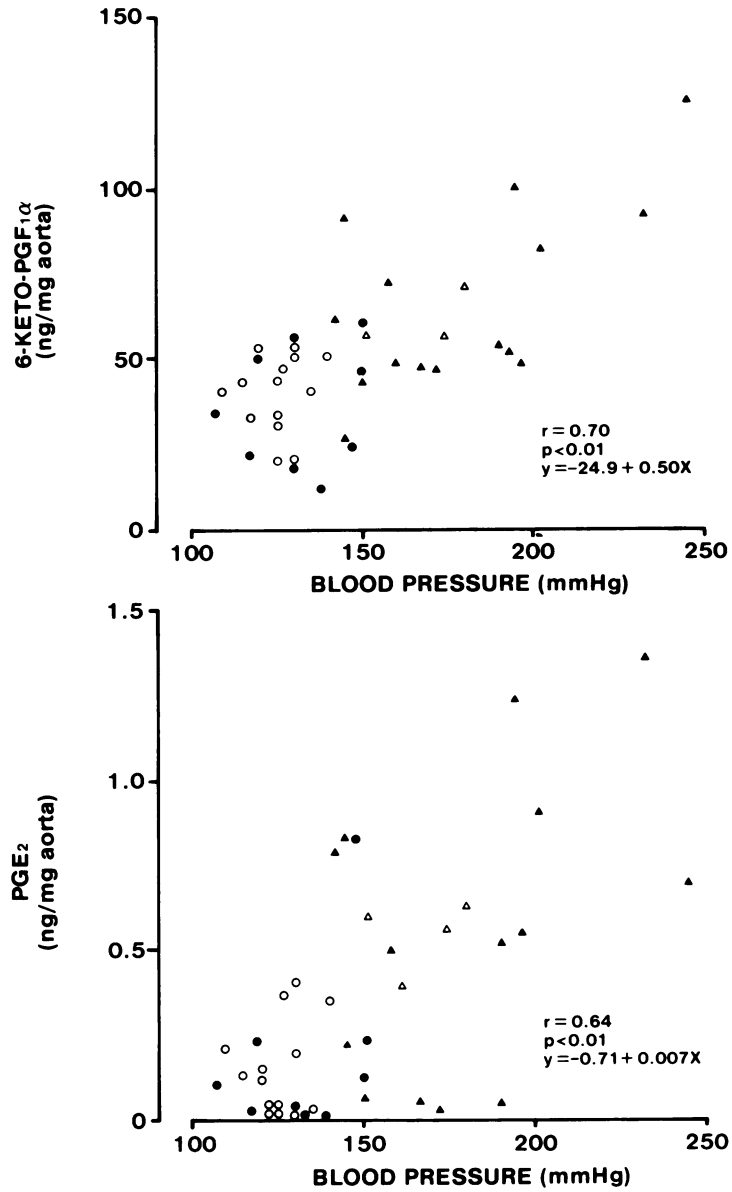

FIGURE 5 Relationship between the systolic blood pressure measured on the 11th d of angiotensin II (AII) or vehicle infusion and the arterial plasma concentrations of immunoreactive 6-keto-$PGF_{1\alpha}$ and PGE_2 measured on the 12th d of infusion. ○: vehicle; ●: AII 75 ng/min; △: AII 125 ng/min; ▲: AII 200 ng/min.

giotensin II administration. Urinary 6-keto-$PGF_{1\alpha}$ may arise both from glomerular ultrafiltration of plasma (19) and from several renal cell types (20, 21). Therefore, the augmentation of urinary 6-keto-$PGF_{1\alpha}$ excretion may have resulted from both stimulation of renal PGI_2 synthesis and elevation of the plasma concentration of PGI_2 and/or 6-keto-$PGF_{1\alpha}$. In this regard we found that slices of inner medulla from rats infused with angiotensin II for 12 d released more 6-keto-$PGF_{1\alpha}$ than did slices from vehicle-infused rats. We also found that the arterial plasma concentration of 6-keto-$PGF_{1\alpha}$ increased with administration of angiotensin II.

That chronic angiotensin II infusion raised the plasma level of 6-keto-$PGF_{1\alpha}$ in rats is consistent with reports that the blood concentration of a PGI_2-like substance increased during short-term angiotensin II infusion in dogs (10, 22, 23); the increase in blood PGI_2

TABLE II

Net Release of 6-Keto-PGF$_{1\alpha}$ and PGE$_2$ from Slices of Renal Cortex and Inner Medulla Incubated in Krebs Solution for 15 min

	6-Keto-PGF$_{1\alpha}$		PGE$_2$	
	Cortex	Medulla	Cortex	Medulla
		ng/mg		
Vehicle (n = 5)	0.05±0.01	7.92±1.09	0.17±0.02	91.97±7.08
AII, 125 *ng/min* (n = 4)	0.06±0.01	18.36±2.49*	0.14±0.02	67.52±13.29
AII, 200 *ng/min* (n = 5)	0.06±0.01	13.53±1.46*	0.23±0.02	76.59±15.93

Values are means±SE, n = number of observations, the asterisk denotes significant difference from value used in vehicle-infused rats ($P < 0.05$); angiotensin II (AII) or vehicle were infused for 12 d before the study.

in dogs was attributed to stimulation of PGI_2 synthesis in the lungs (10, 22) and kidneys (22, 23). The plasma level of 13,14-dihydro-15-keto-PGE_2 increased with administration of angiotensin II suggesting stimulation of PGE_2 tissue production. Metabolism of PGE_2 to 13,14-dihydro-15-keto-PGE_2, which occurs readily in the lung and in other tissues, may have prevented the arterial plasma concentration of PGE_2 from rising during angiotensin infusion (24).

In this present study we found that rings of aorta from rats infused with angiotensin II for 12 d released more 6-keto-PGF$_{1\alpha}$ than did rings from vehicle-infused rats, suggesting augmentation of arterial vascular PGI_2 synthesis in rats receiving angiotensin II. However, PGI_2 synthesis by the aorta need not be representative of that by other vascular segments, in particular the microvasculature. Although rings of aorta from angiotensin II-infused rats also released more PGE_2 than did rings from vehicle-infused rats, it should be noted that the amount of PGE_2 released was <1% of the amount of 6-keto-PGF$_{1\alpha}$ released.

Overall, our data on the urinary excretion, plasma concentration, and in vitro tissue release of 6-keto-PGF$_{1\alpha}$ suggest that augmentation of renal and extrarenal PGI_2 production and, presumably, plasma PGI_2 concentration is a feature of the response of the intact rat to chronic infusion of angiotensin II. Information derived from acute studies indicates that the action of angiotensin II to increase PG synthesis relates to stimulation of phospholipid deacylation (25), which increases the free arachidonic acid available to cyclooxygenase and, consequently, the formation of endoperoxide intermediates and PG. Induction of PG synthesis by angiotensin is characterized by rapidity of onset and reversibility after removal of the stimulus (25). Therefore, it is intriguing that in the present study angiotensin II did not increase the urinary excretion of 6-keto-PGF$_{1\alpha}$ or the release of 6-keto-PGF$_{1\alpha}$ from rings of aorta until after a lag period of several days. Furthermore, under in vitro conditions that did not preserve a critical feature of the in vivo environment, i.e., high angiotensin II levels, rings of aorta and renal medullary tissue obtained from rats infused with angiotensin II for 12 d still released more 6-keto-PGF$_{1\alpha}$ than did corresponding tissues from vehicle-infused rats. These observations require consideration of the possibility that during chronic angiotensin II infusion the enhancement of urinary excretion and in vitro tissue release of 6-keto-PGF$_{1\alpha}$ relates to one or more slowly developing and persistent alterations in function and/or tissue structure rather than to a direct, immediate, and rapidly reversible action of angiotensin II on the phospholipid-arachidonate-prostaglandin system. In this respect, we found that angiotensin II caused a progressive elevation of blood pressure, the final level of which correlated positively on the 12th but not on the 4th d of infusion with the net release of 6-keto-PGF$_{1\alpha}$ from aortic rings in vitro. Several other investigators have also noted an association between high blood pressure and increased vascular PG synthesis (26, 27). Similarly, various features of renal PG metabolism have been found to be abnormal in other animal models of hypertension (28–30). In our study, angiotensin II also produced substantial alterations in renal function, caloric intake, and electrolyte balance that conceivably could have contributed to the overall effect of the peptide on PG production. For example, there are reports that the renal synthesis of PGE_2 is affected by changes in the intake of sodium (31) and potassium (32), and that the vascular synthesis of PGI_2 depends on extracellular potassium concentration (33).

That chronic infusion of angiotensin II in the rat resulted in sustained hypertension confirms a recent report (34). The tendency for the difference between sodium intake and urinary sodium excretion to fall during angiotensin administration at 200 ng/min suggests that renal sodium retention was reduced; this,

and the associated rise in urine volume, may be related to pressure-induced diuresis and natriuresis.

This study was not designed to assess the functional significance of the increase in PG associated with angiotensin II-induced hypertension. However, it is well recognized that several PG are capable of affecting vascular and renal mechanisms involved in blood pressure regulation (35). For example, both PGI_2 and PGE_2 dilate resistance blood vessels and attenuate vascular reactivity to angiotensin II and other pressor stimuli (6). It follows, then, that increased PG production during chronic angiotensin II infusion may be part of a homeostatic response that acts to minimize the angiotensin II-induced blood pressure elevation.

In conclusion, our study shows that chronic angiotensin II infusion in rats produces a progressive increase in blood pressure associated with augmentation of plasma concentration, urinary excretion, and net in vitro release of 6-keto-$PGF_{1\alpha}$ from rings of aorta and slices of renal inner medulla. We found no evidence of increased renal PGE_2 production during angiotensin II infusion. On the basis of these findings, we conclude that augmentation of PGI_2 production is a feature of the response to angiotensin II-induced hypertension.

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