Subtype 2 of Angiotensin II Receptors Controls Pressure-Natriuresis in Rats

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Abstract

Angiotensin II recognizes two receptor subtypes, AT_1 and AT_2, both of them having been recently cloned. Although AT_2 receptors represent 5–10% of angiotensin II receptors in the kidneys of adult rats, their function remains unknown. In the present work, we examined the possible contribution of AT_2 receptors to the regulation of pressure-natriuresis in anesthetized rats infused either with the specific AT_2 antagonist PD 123319, or with CGP 42112B, an AT_2 ligand with agonistic properties. The effects of PD 123319 were examined in a preparation with stable levels of angiotensin II, and in which AT_1 receptors were blocked by the specific antagonist losartan. The effects of CGP 42112B were studied in rats deprived of endogenous angiotensin II. AT_2 receptor blockade with PD 123319 did not change the renal blood flow while it increased the diuresis and natriuresis. These effects persisted even after full AT_1 receptor blockade with losartan. CGP 42112B did not modify the renal blood flow, but dose-dependently decreased urine flow and natriuresis. These results show that, contrary to AT_1 receptors, renal AT_2 receptors have no effect on total renal blood flow, but blunt the pressure-natriuresis, thus demonstrating that this receptor subtype is involved in a function of importance for body fluid and blood pressure regulation. (*J. Clin. Invest.* 1995. 95:1394–1397.) Key words: renin-angiotensin system • renal perfusion pressure • renal blood flow • glomerular filtration rate • sodium excretion

Introduction

One of the most important effects of the renin–angiotensin system is to influence renal functions through the vasoconstrictor and anti-natriuretic properties of angiotensin II (1, 2). As a consequence, either acutely (3) or chronically (4), angiotensin II shifts to the right the pressure-natriuresis relationship which is of utmost importance in the long-term control of blood pressure (5). Binding experiments demonstrated that angiotensin II recognizes two receptor subtypes (6), AT_1 and AT_2,[1] both of them having been recently cloned (7–10). Although AT_2 receptors represent 5 to 10% of angiotensin II receptors in the kidneys of adult rats (11, 12), their function remains unknown as all the so far studied effects of angiotensin II are blocked by AT_1 receptor antagonists (13–15). The aim of the present work was to examine the possible contribution of AT_2 receptors to the regulation of pressure-natriuresis in rats.

Methods

Animals. 10-wk-old male Sprague-Dawley rats (Iffa-Credo, Les Oncins, France) were used in these studies. The rats were allowed at least 1 wk to acclimatize to our laboratory, and were housed in controlled conditions (temperature: $21\pm1°C$; humidity: $60\pm10\%$; lighting: 8–20 h). They were fed a standard rat chow containing 0.3% NaCl (Elevage UAR, Villemoisson sur Orge, France) and tap water ad libitum.

Surgical preparation. Pressure-natriuresis was studied using the method of Roman and Cowley (16). The right kidney and adrenal gland were removed and the rats allowed 7–10 d to recover. On the day of experiment, the rats were anesthetized with Inactin (100 mg/kg, i.p., Byk-Gulden, Constance, Germany) and placed on a heating blanket (Model 50-6980; Harvard Apparatus, Edenbrige, KY) to maintain the rectal temperature at $37\pm0.5°C$. After tracheostomy, the left jugular vein was cannulated for infusion. Catheters were placed into the left carotid and femoral arteries to sample blood and to record the mean arterial blood pressure through a pressure transducer (Model P23ID; Statham Instrument Division, Gould Inc., Cleveland, OH). After an abdominal incision, the left kidney was exposed and denervated by stripping all the visible renal nerves and coating the renal artery with a 10% solution of phenol in ethanol. The remaining adrenal gland was then removed and the ureter cannulated for urine collection. Two adjustable silastic balloon cuffs were placed around the aorta, one above the renal artery between the superior mesenteric and celiac arteries, the other below the left renal artery so that the renal perfusion pressure could be fixed at different levels. Silk ligatures were placed loosely around the superior mesenteric and celiac arteries and tighten to further elevate the renal perfusion pressure. An ultrasonic flow probe (1R85) placed around the left renal artery allowed to continuously record the renal blood flow using a transonic transit-time flowmeter (Model T106; Transonic Systems Inc., Ithaca, NY). After a priming dose (250 mg/kg, i.v.) of polyfructosan (Inutest, Laevosan, Linz, Austria), a hormone-cocktail (3) designed to fix the circulating levels of the most important sodium- and water-retaining hormones was infused at a rate of 330 $\mu l/kg$ per min (Pump Model 2400-001; Harvard Apparatus, South Natick, MA). It contained D-aldosterone (66 ng/kg per min), hydrocortisone (33 ng/kg per min), norepinephrine (333 ng/kg per min) and Arg^8-vasopressin

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1. *Abbreviations used in this paper:* ACE, angiotensin converting enzyme; AT1 and AT2 receptors, subtypes 1 and 2 of angiotensin II receptors.
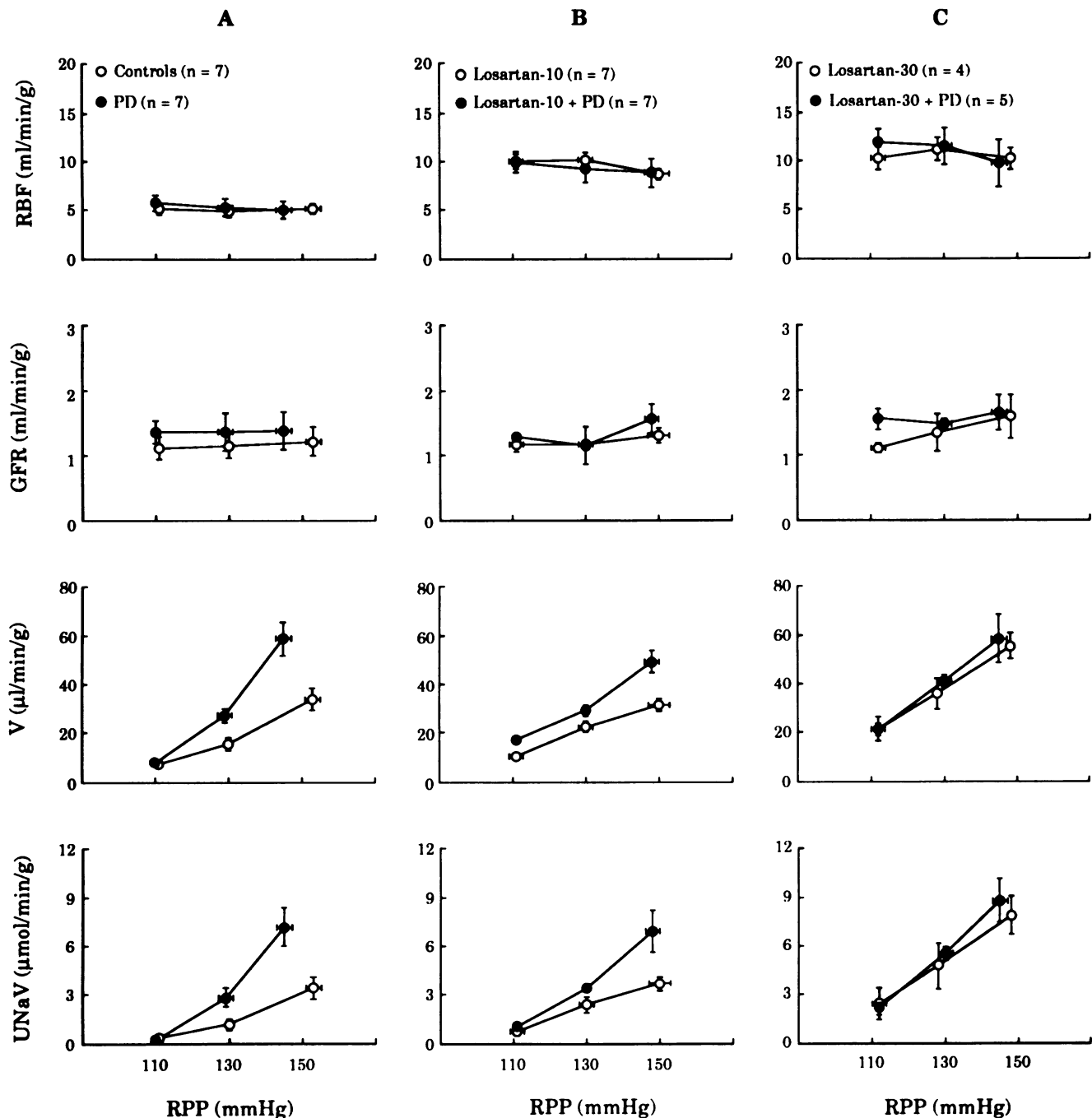
Figure 1. Effects of AT_2 and combined AT_1 and AT_2 receptor blockade on the relationships between renal perfusion pressure (*RPP*), renal blood flow (*RBF*), glomerular filtration rate (*GFR*), urine flow (*V*), and natriuresis (*UNaV*) in rats with fixed plasma concentrations of angiotensin II. (*A*) Controls and PD 123319 (PD, 50 μg/kg per min i.v.). (*B*) Losartan, 10 mg/kg i.v. given alone (*Losartan-10*) or with PD 123319 (*Losartan-10 + PD*). (*C*) Losartan, 30 mg/kg i.v. given alone (*Losartan-30*) or with PD 123319 (*Losartan-30 + PD*).

acetate (0.17 ng/kg per min). Drugs were obtained from Sigma Chemicals Co. (St. Louis, MO) and dissolved in 0.9% sodium chloride containing 1% bovine serum albumin (Fraction V; Sigma Chemicals Co.) and 1.25% polyfructosan. At the end of the experiment, the kidney was decapsulated, removed, cut in half, blotted dry and weighed.

Renal function parameters. Renal perfusion pressure (mmHg) was estimated as the femoral artery pressure when the suprarenal aortic cuff was inflated and as the carotid artery pressure when the infrarenal aortic cuff was inflated. Glomerular filtration rate (ml/min per g) was measured by polyfructosan clearance. Urine flow (μl/min per g) was determined by weighing, and sodium concentration measured by flame pho-

tometry (IL meter, model 243; Lexington, MA) so as to calculate the urinary sodium excretion (μmol/min per g). All the renal function parameters were normalized per gram of the left kidney weight.

Experimental protocols. In the first protocol, the circulating levels of angiotensin II were maintained stable by an intravenous injection of quinapril (10 mg/kg, i.v.), an angiotensin converting enzyme (ACE)[1] inhibitor (Parke-Davis, Ann Arbor, MI) given 35 min before the start of the study followed by an intravenous infusion of angiotensin II (30 ng/kg/min, i.v.). PD 123319 (Parke-Davis, Ann Arbor, MI), a specific AT_2 receptor antagonist (IC_{50} of 2×10^{-8} M and above 1×10^{-4} M for AT_2 and AT_1 receptors respectively) (17) was infused at the dose of 50
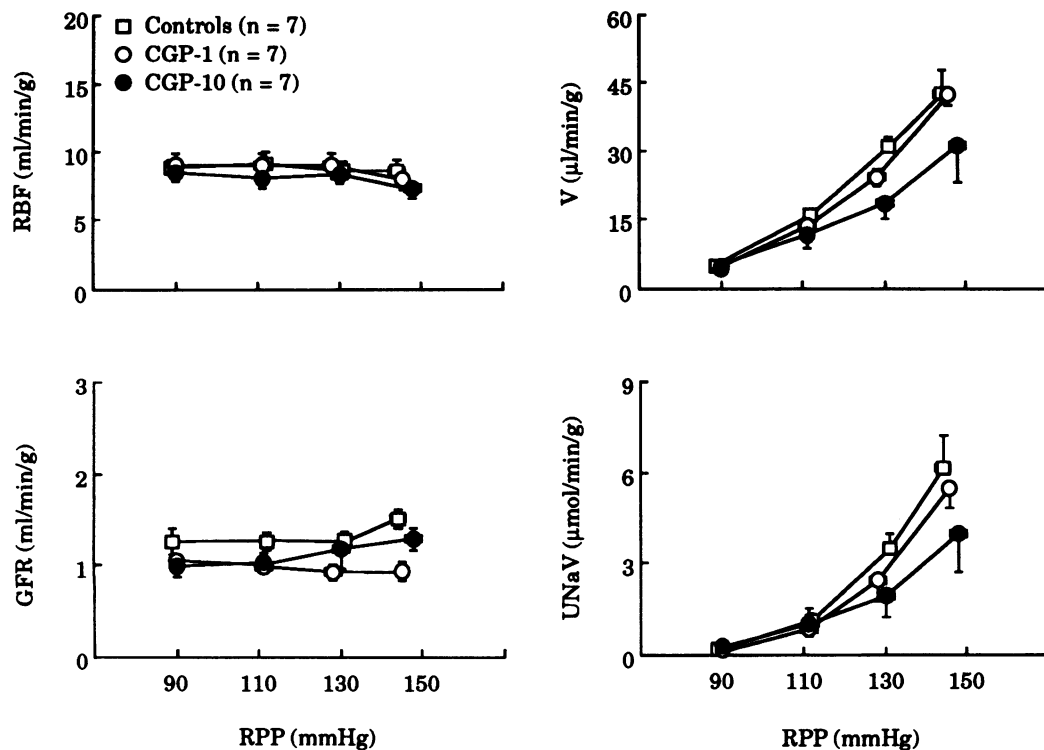
Figure 2. Effects of CGP 42112B on the relationships between renal perfusion pressure (*RPP*), renal blood flow (*RBF*), glomerular filtration rate (*GFR*), urine flow (*V*) and natriuresis (*UNaV*) in rats deprived of endogenous angiotensin II (*Controls*). CGP 42112B was given i.v. at the doses of 1 (*CGP-1*) or 10 (*CGP-10*) µg/kg per/min.

µg/kg per min which, according to Macari et al. (18), yielded plasma concentrations near 3×10^{-6} M, i.e., a value which remains highly specific for AT_2 receptors. However, to further exclude any effect of PD 123319 on AT_1 receptors, the experiment was repeated in rats having received a single intravenous injection of losartan (Du Pont Merck Pharmaceutical Co., Wilmington, DE), a long-acting antagonist of AT_1 receptors (IC_{50} of 3×10^{-4} and 7×10^{-9} M for AT_2 and AT_1 receptors, respectively). Losartan was given 30 minutes prior to the experiment, either at a usual (10 mg/kg) or a high (30 mg/kg) dose, which led to plasma concentrations near 6×10^{-5} and 3×10^{-4} M respectively (18). In the second protocol, the effects of CGP 42112B (Ciba-Geigy Ltd, Basel, Switzerland), a specific AT_2 receptor ligand (IC_{50} of 5×10^{-10} and 2×10^{-6} M for AT_2 and AT_1 receptors respectively) which exhibits agonistic properties (10) were studied in a preparation deprived of endogenous angiotensin II by an intravenous injection of quinapril (10 mg/kg, i.v.). Two different doses (1 and 10 µg/kg per min, i.v.) of CGP 42112B were used, which yielded plasma concentrations of 1×10^{-8} and 4×10^{-8} M, respectively (18). Both studies were conducted in agreement with our institutional guidelines for animal care.

Statistical analysis. Values are mean ± standard error. One-way ANOVA was used to assess the effects of renal perfusion pressure within groups, and two-way ANOVA for between-group comparisons.

Results

As shown in Column A of the Fig. 1, in control conditions, an increase in the renal perfusion pressure from 110 to 150 mmHg, did not change the renal blood flow and glomerular filtration rate but induced an eightfold increase in the natriuresis. The infusion of PD 123319 did not change the renal blood flow and glomerular filtration rate, while it significantly increased ($P < 0.001$) increased urine flow and natriuresis, especially at the two highest renal perfusion pressure levels. Column B shows that, when compared to controls, the 10 mg/kg dose of losartan almost doubled the renal blood flow while it did not affect significantly

the glomerular filtration rate as well as the relationships between pressure, urine flow and natriuresis. When PD 123319 was added to losartan, renal blood flow and glomerular filtration rate remained unchanged while urine flow ($P < 0.001$) and natriuresis ($P < 0.01$) significantly increased. As shown in Column C, a 30 mg/kg dose of losartan did not elevate renal blood flow more than the 10 mg/kg dose but, in addition, significantly enhanced the pressure-natriuresis. When PD 123319 was added to this dose of losartan it was devoid of effects.

As CGP 42112B exhibits agonistic properties, its effects were studied in rats deprived of endogenous angiotensin II. Fig. 2 shows that in control animals, a stepwise increase in renal perfusion pressure did not change renal blood flow and glomerular filtration rate, but increased urine flow and natriuresis to a similar extent as that observed in the first experiments after infusion of PD 123319. In this preparation, CGP 42112B did not modify the renal blood flow and glomerular filtration rate, but dose-dependently decreased urine flow and natriuresis. These changes reached statistical significance ($P < 0.01$ and $P < 0.05$ for urine flow and natriuresis respectively) with the 10 µg/kg per min dose.

Discussion

The present work demonstrates that in rats receiving a normal sodium diet, acute stimulation of renal AT_2 receptors has no effect on total renal blood flow but blunts the pressure-diuresis and natriuresis. Previous attempts to disclose a function for these receptors suggested that AT_2 antagonists could elevate the urine volume, chloride and bicarbonate excretion in anesthetized rats (19) or increase free water formation in anesthetized dogs (20). However, the specificity of these observations was questioned (18, 21) as the doses of the compounds used to

block the AT_2 receptors were high enough to non-specifically interact with AT_1 receptors. In the present work, the doses of antagonists and agonist used were maintained low enough to remain highly specific for AT_2 receptors. In these conditions, AT_2 receptor stimulation or blockade dose-dependently reduced or enhanced the pressure-natriuresis. This effect persisted after administration of 10 but not of 30 mg/kg of losartan, a difference which is likely to be due to a non-specific blockade of AT_2 receptors by the high dose of losartan since: (a) the maximal vasodilation and thus the complete AT_1 blockade was already obtained with the 10 mg/kg dose, and (b) according to Macari et al. (18), the plasma concentration of losartan following the 30 mg/kg dose might reach the IC50 of this drug for rabbit uterus AT_2 receptors.

Such an observation is in good agreement with two recent reports showing that losartan given either acutely (22) or chronically (23) does not affect the pressure-natriuresis or the renal adaptation to a low sodium diet in rats. Since this lack of effect of losartan is in contrast with the natriuretic properties of ACE inhibitors (23, 24), it supports a role for AT_2 receptors as promoters of sodium reabsorption as ACE inhibitors which suppress angiotensin II formation, suppress also the stimulation of both AT_1 and AT_2 receptors.

In addition, it is noteworthy that the effects of both the antagonist (PD 123319) and the ligand with agonistic properties (CGP 42112B) were more marked when the renal perfusion pressure was elevated than when it remained near baseline. This suggests that part of the failure of previous attempts to disclose a function for renal AT_2 receptors could have been due to the fact that, in these experiments, renal perfusion pressure was either normal or uncontrolled. The observation that AT_2 receptors were found more efficient at high than at normal pressure indicates that they may directly interact with the mechanisms involved in the pressure-natriuresis. Despite extensive work, these mechanisms are not fully understood. The two major current hypothesis rely upon a blood pressure-dependent increase in renal interstitial pressure (25) or in the medullary blood flow (26). Therefore the location and the site (tubular or vascular) of action of AT_2 receptors remains to be determined. However, as far as the present data can fully be extrapolated to physiological conditions, it can be speculated that besides the role of AT_1 receptors, angiotensin II through AT_2 receptors stimulation is able to prevent the sodium losses which otherwise would accompany any acute increase in blood pressure. In addition, since an abnormal pressure-natriuresis is the hallmark of any form of chronic hypertension (5), an enhanced role of AT_2 receptors could well be involved in the development and/or the maintenance of hypertension.

In conclusion, it appears that, in rats fed a normal sodium diet, AT_2 receptor stimulation acutely blunts the diuretic and natriuretic responses to blood pressure increases. This effect is likely to contribute to the role of the renin-angiotensin system in body fluid and blood pressure regulation.

Acknowledgments

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