Blockade of AT_1 Receptors by Losartan did not Affect Renin Gene Expression in Kidney Medulla

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Abstract. This study was designed to determine particular changes in the renin gene expression and activity in renal cortex and medulla after AT_1 receptor blockade. It was found that two-week-treatment with AT_1 blocker losartan induced an increase in tissue renin activity in both parts of kidney causing subsequent elevation of plasma renin activity. Renin mRNA in losartan-treated rats was increased only in cortex, suggesting cortex origin of elevated renin activity in medulla. Medullary renin mRNA indicated local synthesis of renin within the whole kidney and supported the idea of the presence of tissue renin-angiotensin system. Our results show that gene expression of renin in kidney medulla is insensitive to AT_1 receptor blockade and this points out that the regulation of kidney renin-angiotensin system probably differs from that in cortex.

Key words: Renin — AT_1 receptor — Losartan — Kidney cortex — Kidney medulla

Introduction

It has been demonstrated that the kidney contains functional and independently regulated tissue renin-angiotensin system (RAS) (Levens et al. 1981; Kastner et al. 1984; Dzau and Ingelfinger 1989), which is supported by the fact that the concentrations of angiotensin II (Ang II) in proximal tubule fluid are about 100–1000-fold higher than in plasma (Braam et al. 1993). Furthermore, all RAS components necessary for Ang II formation have been detected in the kidney (Ingelfinger et al.

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1990; Johnston et al. 1993; Chen et al. 1994; Harris and Gomez 1997). The renin synthesis and storage in mature kidney is primarily located to juxtaglomerular apparatus (Gomez et al. 1989). However, the presence of renin mRNA and protein has been also determined in proximal tubule (Chen et al. 1994; Henrich et al. 1996), in distal nephron cells (Gilbert et al. 1999; Prieto-Carrasquero et al. 2004) and in dissected outer and inner medulla (Shin et al. 1999).

The intrarenal Ang II has potent effects on renal function. These actions include modulation of renal blood flow, glomerular filtration rate, tubular epithelial transport, renin release and cellular growth (de Gasparo and Levens 1994). Ang II effects are mediated by binding to Ang II receptors (the AT_1 and AT_2 subtypes), which also have been detected in the kidney (Zhuo et al. 1992; de Gasparo and Levens 1994; Ozono et al. 1997; Miyata et al. 1999).

The plasma Ang II downregulates renal renin gene expression during normal development *via* AT_1 receptor subtype (Schunkert et al. 1992; Turfo-McReddie et al. 1994). The blockade of the AT_1 receptor with specific nonpeptide antagonist, losartan, elicited an increase in plasma renin activity (PRA) and renin mRNA (Pals and Couch 1993; Turfo-McReddie et al. 1994) and also elevation of plasma levels of Ang II (Kasper et al. 2005). On the other hand, recent findings have proven involvement of AT_2 receptors in regulation of kidney RAS activity through AT_2 receptors *via* inhibition of renin synthesis (Siragy et al. 2005).

We have previously shown, that the responses of plasma and kidney RAS to angiotensin-converting enzyme inhibition are different suggesting their, at least in part, independence (Grima et al. 1997). Furthermore, results of Prieto-Carrasquero et al. (2004) suggest that regulation of renin gene expression under high Ang II state differs in kidney cortex and medulla. In the medulla, renin gene expression is not inhibited by Ang II. Therefore, our study was designed to determine the effect of AT_1 receptor blockade by losartan on particular changes in the renin activity and renin mRNA levels in kidney cortex and medulla.

Materials and Methods

Animals

Male adult Wistar-Kyoto rats (obtained from Iffa-Credo, l'Arbresle, France) were housed at 23 ± 2 °C and 12 h light/dark cycle with free access to water and rat chow (UAR, Epinay sur Orge, France). Adult rats were divided into 2 groups. Rats in one group were receiving losartan in dose 30 mg/kg/day (efficacy dose for antihypertensive effects). It was given by single daily oral gavage during 14 days ($n = 10$). The control group ($n = 9$) and rats were receiving an equal amount of distilled water. Blood pressure was measured by using indirect tail cuff sphygmomanometry (Physiograph Desk model DMP-4A, Narcobio systems, Inc., USA) as described van den Berg et al. (1990). All rats were trained three times in similar conditions during one week, in a quiet room. Blood pressure was measured the day before rats were killed, 3 h after the gavage corresponding to blood maximal

concentration of losartan. Animals were starved the night before plasma and tissue sampling. and they were killed 3 h after the last gavage by decapitation. Principle of laboratory animal care and all procedures were in accordance with the guidelines of the French government and the European Community concerning the use of animals scientific research.

Plasma and kidney renin activity

After decapitation, trunk blood was rapidly collected in ice-cold EDTA tubes. The blood was promptly centrifuged at 4 ℃ for 10 min at 2000 × *g* and the plasma was frozen in liquid nitrogen and stored at −80 ℃ until assay.

After decapitation, the left kidney was rapidly removed and dissected on an ice plate into cortex and medulla and was used for determination of kidney renin activity. Tissues were homogenized in a phosphate buffer with 144 mmol/l phenylmethylsulphonyl fluoride and 10 mmol/l phenylmercuric acetate (in ethanol) and centrifuged at 4 ℃ for 20 min at 5000 × *g*. The supernatants were stored at −20 ℃ until assay.

PRA and kidney renin activity (KRA) were evaluated indirectly by determining the amount of generated Ang I quantified by radioimmunoassay (RIA). PRA was measured after 30 min incubation of plasma at 37 ℃ in the presence of 5 mmol/l 8-hydroquinoline (Ingert et al. 2002). KRA was measured after 30 min incubation of diluted supernatants in the presence of plasma enriched in angiotensinogen obtained from rats 48 h after binephrectomy (Campbell et al. 1991). Polyclonal rabbit anti-Ang I serum diluted at 1/60,000 (cross reactivity with Ang II: <0.4 %, with Ang III: <0.4 %) was used to quantification of Ang I by RIA (Nussberger et al. 1986). For reactions, 250 μl of rat plasma or 40 μl of renal medulla and cortex diluted homogenate were used. A parallel series of incubation was performed at 4 ℃ to provide a control for "nonspecific" interference in the RIA for Ang I. PRA and KRA are expressed as nanograms of Ang I generated *per* milliliter of plasma or micrograms of Ang I generated *per* gram of tissue weight *per* hour, respectively.

Total RNA isolation and semi-quantitative reverse transcription-PCR

After decapitation, the right kidney was also dissected into cortex and medulla and rapidly frozen in liquid nitrogen and stored until assay (−80 ℃). Total RNA was isolated from these tissues using guanidinium-thiocyanate/phenol chloroform extraction method according to Chomczynski and Sacchi (1987). 2 μg of total RNA were reversely transcribed using Ready-To-Go You-Prime First-Strand Beads kit and pd$(N)_6$ random hexamer primers (Amersham Pharmacia Biotech). Polymeraze chain reaction (PCR) amplification was performed in a total volume of 25 μl containing 250 μmol/l deoxyribonucleotide triphosphate, 1U DyNAzyme^{TM} II DNA polymerase (Finnzymes, Finland), 2 μl of first strand cDNA and 12.5 pmol of each primers for renin gene 5′-TCT CAG CAA CAT GGA CTA TGT GC-3′ (sense) and 5′-TTA GCG GGC CAA GGC GAA CC-3′ (antisense) yielding a 190 bp size product. Reactions were normalized using housekeeping gene β-actin: 5′-AGT GTG ACG TTG ACA-3′ (sense) and 5′-GAC TGA TCG TAC TCC TGC-3′

(antisense) yielding a 240 bp size product. PCR reaction mixture was denatured to 94 ℃/5 min and allowed to proceed for 35 cycles (denaturation 94 ℃/1 min, annealing 56 ℃/1 min, extension 72 ℃/1 min) and final extension was performed at 72 ℃ for 7 min (Pinterova et al. 2000). The number of cycles was determined in order to be within linear range of amplification and it was also verified that the quantity of PCR product is directly proportional to the amount of cDNA used. Specific PCR products were separated by electrophoresis in 2 % agarose gels in the presence of ethidium bromide stain. The band intensities were quantified by optical densitometry using UltraLum KS-4000 camera (Ultra-Lum, Inc., Canada) and 1D Image Analysis software (Eastman Kodak).

Statistical analysis

The results are expressed as the mean ± SEM. Statistical comparisons were made using one-way ANOVA.

Results

To characterize our experimental animals, their blood pressure and PRA were measured (Tab. 1). Specific blockade of AT_1 receptor by losartan resulted in a significant decrease in blood pressure in comparison with control rats. In accordance with other studies (Kasper et al. 2005), losartan-treated rats showed an increase in PRA (17 times in our experiment).

Table 1. Plasma renin activity (PRA) and blood pressure in rats treated with losartan and in control rats

	Blood pressure (mm Hg)	PRA (ng Ang I/ml/h)	n
Controls	120 ± 2	19.87 ± 2.96	9
Losartan	104 ± 3 ***	351.14 ± 20.67 ***	10

The systolic blood pressure was measured 3 times and the average of these measurements was taken. *** $p < 0.001$ *vs.* controls.

KRA was measured separately in cortex and medulla. In line with elevated PRA, the KRA in both parts of kidney was increased in rats receiving losartan (Fig. 1) confirming our previous data (Ingert et al. 2002). KRA in renal cortex was elevated by 68 % and in medulla by 176 %. Medullar KRA represented about 35 % in controls and 60 % in losartan-treated animals of the KRA levels in corresponding cortex groups.

The presence of renin mRNA was detected in kidney cortex as well as in medulla. Renin gene expression remarkable differed between cortex and medulla in response to AT_1 receptor blockade (Fig. 2). The renin mRNA level was significantly

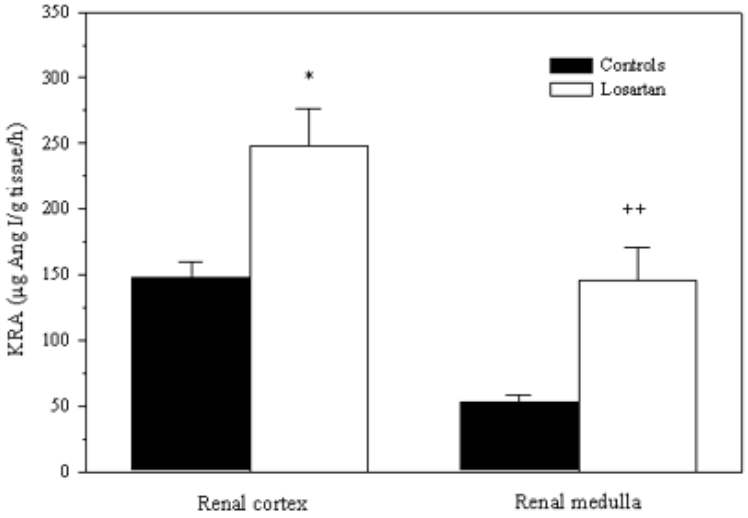

Figure 1. Difference in kidney renin activity (KRA) between control and losartan-treated rats within renal cortex (* $p < 0.05$ *vs.* control) and medulla (++ $p < 0.01$ *vs.* control).

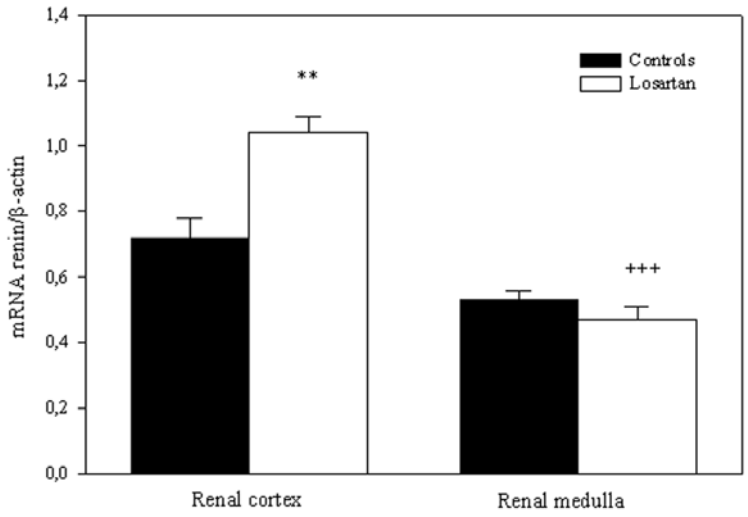

Figure 2. Different gene expression of renin in renal cortex and medulla of the kidney in losartan-treated animals (+++ $p < 0.001$) and between control and losartan-treated rats in cortex (** $p < 0.01$).

elevated (by 44%) after blockade in renal cortex. However, losartan did not exert stimulatory effect on the renin gene expression in the medulla.

Discussion

Renin synthesis and production is generally limited to the juxtaglomerular cells in the cortex, but a few studies suggest its formation in tubular segments, too (Chen et al. 1994; Henrich et al. 1996). We detected the presence of renin mRNA in renal medulla. Our results raised the question of the origin of renin. We hypothesized that medullary renin mRNA might originate from the collecting ducts where renin expression was already described (Prieto-Carrasquero et al. 2004). Alternatively, the presence of renin mRNA might be caused by tissue cross contamination during kidney dissection but in such case, similar pattern of responses to losartan would be expected in both cortex and medulla.

It is known that Ang II *via* phospholipase C activation mobilizes Ca^{2+} from internal stores in juxtaglomerular cells and this increase in the calcium concentration inhibits the exocytosis of renin (Schweda et al. 2000). It has been also described that Ang II exerts direct feedback inhibition on juxtaglomerular renin gene expression *via* AT_1 receptors (Schunkert et al. 1992; Turfo-McReddie et al. 1994). Specific blockade of AT_1 receptor caused elevation of PRA (Pals and Couch 1993) with subsequent elevation of plasma Ang II (Kasper et al. 2005). Based on our results, we confirm that the increase in PRA after losartan treatment is a consequence not only of stimulated renin secretion but also of elevated renin gene expression in renal cortex.

A different unexpected situation was observed in renal medulla, where KRA, but not the renin gene transcript was increased after AT_1 receptor blockade. As renin gene expression in the renal medulla was insensitive to losartan, we inclined to assumption that the regulation of tubular renin differed from that of juxtaglomerular renin. Prieto-Carrasquero et al. (2004) have supposed that activity of medullary distal nephron renin is probably a contributing factor to intratubular and intrarenal formation of Ang II. We suggest that elevated medullary KRA after losartan treatment, found in our experiment, might originate from cortex because specific binding sites, which bind both circulatory and interstitial renin have also been described in the medulla (Sealey et al. 1996). This suggestion is strengthened by the fact that the renin bound to the renin/prorenin receptor posses four times increased catalytic efficiency of angiotensinogen conversion to Ang I (Nguyen et al. 2002) what might also possibly contribute to elevated KRA in medulla. Furthermore, renin may mimic some intracellular actions of Ang II independently of Ang II.

The intrarenal effects of Ang II are mediated to a substantial extent by AT_1 receptors (Wang and Li 2000). AT_1 receptors were described throughout the kidney with the highest density in vasculature of the renal cortex, glomeruli and in the outer medulla. Their presence in the inner medulla was associated with collecting ducts (Zhuo et al. 1992; Healy et al. 1995; Harrison-Bernard et al. 1997; Miyata et al. 1999; McDougall et al. 2000; Wang and Li 2000). Ang II directly stimulates epithelial Na^+ channel activity in the cortical collecting duct *via* AT_1 receptors (Peti-Peterdi et al. 2002). Divergent regulation of circulating and intrarenal RAS

has been shown (Grima et al. 1997; Kasper et al. 2005) and it seems that medulla is involved in regulation of intrarenal RAS (Prieto-Carrasquero et al. 2004) as indicate also our results.

At least two mechanisms might account for insensitivity of renal medullary renin mRNA to losartan treatment. Recent evidence that AT_2 receptors regulate kidney RAS activity *via* inhibition of renin synthesis strengthens their possible involvement in medullary renin regulation (Siragy et al. 2005). However, the presence of AT_2 receptors and their functional role within adult kidney has not been fully elucidated. Studies have indicated that less then 20 %, if any, of the Ang II binding sites can be attributed to the AT_2 receptors located to various tubular and vascular segments from the cortex and medulla (Zhuo et al. 1992; de Gasparo and Levens 1994; Ozono et al. 1997; Miyata et al. 1999; McDougall et al. 2000; Armando et al. 2002; Joly et al. 2005). Second possibility is that medullary AT_1 receptors transduce different signal than those in cortex. This seems to be a case when the receptors are agonist-stimulated (Prieto-Carrasquero et al. 2005).

Nevertheless, we observed one important feature that there is a dissociation in the responses of renin mRNA to losartan treatment between renal cortex and medulla. In the cortex, renin mRNA and KRA increased in parallel and in accordance with PRA while in the medulla, the elevation in KRA was not associated with an increase in renin mRNA. Our results show that medullary sites of renin synthesis are insensible to losartan. The exact mechanism of Ang II-mediated renin gene regulation awaits for more detailed investigation.

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