Protective effects of the angiotensin II type I (AT_1) receptor blockade in low-renin deoxycorticosterone acetate (DOCA)-treated spontaneously hypertensive rats

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ABSTRACT

The present study evaluates the participation of oxidative stress, tissue angiotensin II (Ang II) and endothelin (ET) in the effects of losartan on blood pressure (BP), ventricular hypertrophy and renal injury in spontaneously hypertensive rats (SHRs), and explores how these effects are modified when spontaneous hypertension is transformed in a low-renin model by the administration of deoxycorticosterone acetate (DOCA). The following groups were used: SHR-control, SHR + DOCA, SHR + losartan and SHR + DOCA + losartan. Tail systolic BP was measured once a week. After 9 weeks of treatment, direct BP and metabolic, morphological, biochemical and renal variables were measured. DOCA administration to SHRs produced an increase in BP, ventricular hypertrophy, renal weight, proteinuria, renal histopathological lesions, urinary excretion of isoprostane $F_{2\alpha}$ and ET levels in the renal cortex. Losartan reduced BP, plasma malondialdehyde levels, urinary excretion of isoprostane $F_{2\alpha}$, renal Ang II and renal and urinary levels of ET in the SHR and DOCA-treated SHR groups. Losartan increased plasma nitrite/nitrate in SHRs, but not in low-renin DOCA-treated SHRs. Losartan reduced ventricular hypertrophy and ventricular Ang II in SHRs, but not in DOCA-treated SHRs. Losartan significantly decreased proteinuria and renal injury in DOCA-treated SHRs. We conclude that (i) the DOCA-induced aggravation of hypertension, ventricular hypertrophy and renal injury in SHRs is accompanied by augmented oxidative stress and increased levels of ET in the renal cortex, which could contribute to their development; and (ii) losartan reduced oxidative stress and renal Ang II and ET in SHRs and DOCA-treated SHRs, which might contribute to its antihypertensive and renoprotective effects, regardless of renin status.

Key words: deoxycorticosterone acetate (DOCA), hypertension, losartan, renal injury, spontaneously hypertensive rat.

Abbreviations: Ang II, angiotensin II; AT_1, Ang II type 1 receptor; BP, blood pressure; BW, body weight; DOCA, deoxycorticosterone acetate; ET, endothelin; HA, hyaline arteriopathy; LVW, left ventricular weight; MAP, mean arterial pressure; MDA, malondialdehyde; MH, myointimal hyperplasia; NO, nitric oxide; NOx nitrate/nitrite; PAS, periodic acid Schiff; RAS, renin–angiotensin system; RVW, right ventricular weight; SBP, systolic BP; SHR, spontaneously hypertensive rat.

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INTRODUCTION

Although plasma renin activity and plasma levels of angiotensin II (Ang II) are not increased in spontaneously hypertensive rats (SHRs) [1], there is considerable evidence that the renin–angiotensin system (RAS) plays an important role in the pathogenesis of this experimental model. Thus chronic Ang II type 1 (AT_1) receptor blockade prevents or attenuates the development of hypertension [2,3], cardiac hypertrophy [2] and renal injury [3] in SHRs.

The classical view of Ang II as a vasoactive agent that participates in local and systemic haemodynamic regulation has been recently enlarged, and it is now considered a true cytokine. Tissue Ang II has long been known to play a role in the cardiac hypertrophy [4] and renal injury [5] of hypertension. Tissue endothelin (ET) also participates as a growth factor in the cardiac hypertrophy and renal fibrosis of hypertensive disease [6].

The SHR model is characterized by an increase in oxidative stress [7,8], and increased superoxide anion production is thought to contribute to hypertension in SHRs [7]. In fact, reduction of superoxide anions with alloxanthine, an inhibitor of xanthine oxidase [9] or CuZn superoxide dismutase [7], acutely decreased mean arterial pressure (MAP) in SHRs. Additionally, the 7-day administration of tempol, a membrane-permeant superoxide dismutase mimetic, also reduced blood pressure (BP) in SHRs [10].

Ang II can contribute to the maintenance of hypertension through the stimulation of oxidative stress [11]. The slow pressor response to Ang II is accompanied by increased levels of 8-iso-prostaglandin $F_{2\alpha}$ (isoprostane $F_{2\alpha}$) [12]. Isoprostane $F_{2\alpha}$ is formed non-enzymically from the attack of superoxide radicals on arachidonic acid *in vivo* and *in vitro* [13]. F_2-isoprostanes exert potent vasoconstrictor and antinatriuretic effects [14]. Furthermore, Ang II can enhance ET production [15], which has also been shown to stimulate oxidative stress [16]. These alterations, together with a reduction of nitric oxide (NO), which is quenched by superoxide [17], could potentiate the vasoconstrictor effect of Ang II and may explain how hypertension is maintained when Ang II levels are normal [1]. This sequence of events has been proposed to play a role in the pathophysiology of SHRs [11].

Chronic administration of deoxycorticosterone acetate (DOCA) to NO-deficient hypertensive rats increases BP, suppresses plasma renin activity and makes this type of hypertension resistant to AT_1 receptor blockade [18]. In the DOCA-salt model, the chronic administration of AT_1 receptor antagonists does not reduce BP [19,20], although it ameliorates cardiac hypertrophy [19] and renal injury [20].

The present study was designed to analyse the participation of oxidative stress and tissue Ang II and ET in the effects of AT_1 receptor blockade on BP, cardiac hypertrophy and renal injury in SHRs, and to explore how these effects are modified when spontaneous hypertension is transformed in a low-renin model by the administration of DOCA.

METHODS

Animals

Male SHRs purchased from Harlan Laboratories (Barcelona, Spain) were used. The experiments were performed according to European Union Guidelines for the ethical care of animals. Rats with an initial age of 10 weeks, weighing 250–270 g at the beginning of the study, were randomly assigned to different experimental groups. All animals had free access to standard rat diet with a sodium content of 0.5 % (Rodent toxicology diet; B&K, Barcelona, Spain) and tap water *ad libitum*, except where stated.

Experimental protocol

The rats were divided into four groups: control (SHR-control), DOCA-treated (SHR + DOCA), losartan-treated (SHR + losartan) and losartan plus DOCA-treated (SHR + DOCA + losartan) rats ($n = 8$ in all groups). Losartan was given in the drinking water at a concentration of 20 mg/100 ml, resulting in a daily intake of approx. 14.5 $mg \cdot kg^{-1} \cdot day^{-1}$. The concentration of losartan in the groups was adjusted every 2 days according to their respective fluid intake to ensure that a similar dose was given. DOCA was administered subcutaneously at a dose of 12.5 $mg \cdot rat^{-1} \cdot week^{-1}$. All treatments were started at the same time and were maintained for 9 weeks.

Body weight (BW) and tail systolic BP (SBP) were determined once a week during the course of the experiment. SBP was measured by tail-cuff plethysmography in unanesthetized rats (LE 5001-Pressure Meter; Letica SA, Barcelona, Spain). At least seven determinations were made at every session and the mean of the lowest three values within a range of 5 mmHg was recorded as the SBP level.

After the time-course study, all animals were housed in metabolic cages with free access to food and their respective drinking fluids. After 2 days of adaptation, the food and water intake and urine were gathered during two consecutive days. The values obtained each experimental day were averaged for statistical purposes. The urinary variables measured were diuresis, natriuresis, kaliuresis, F_2-isoprostanes, ET, creatinine and proteinuria.

After the metabolic study was completed, the femoral artery was cannulated. After a 24-h recovery period, direct BP and heart rate were recorded continuously for 60 min. The values obtained during each of the last 30 min were averaged to obtain the mean BP value. Blood samples from the femoral catheter were taken to determine plasma electrolytes, creatinine, total protein, malondialdehyde (MDA), nitrate/nitrite (NOx) and

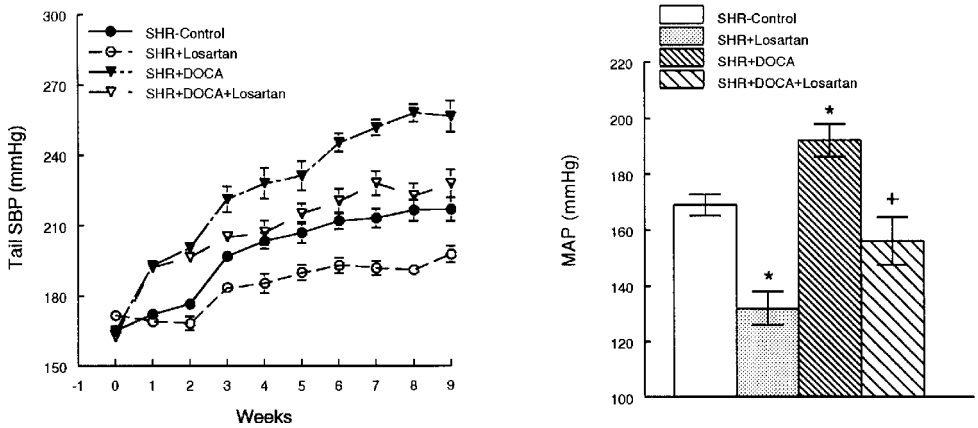

Figure 1 **Time course of SBP (left panel), measured by the tail-cuff method, and final MAP (right panel), measured by direct recording (femoral artery), in conscious rats**

Data are means ± S.E.M. $^{*}P < 0.01$ compared with SHR-control, $+ P < 0.01$ compared with SHR + DOCA.

Ang II. Tissue Ang II and ET were measured in the left ventricle and in the renal cortex and medulla. BW and ventricular and kidney weights were also measured at the end of the study.

Analytical procedures

Plasma Ang II, urinary ET and tissue Ang II and ET were measured in extracted (C_{18} column) samples with the use of RIA kits purchased from EURO-DIAGNOSTICA AB (Malmö, Sweden). Ventricular and kidney tissues were homogenized with the aid of a tissue grinder (Omni International; Warrenton, VA, U.S.A.) at 1200 *g* in ice-cold homogenization buffer [25mM Tris/HCl (pH 7.6), 0.5 mM D,L-dithiothreitol, 10 μg/ml pepstatin, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mM PMSF]. The homogenate was centrifuged 5 min at 3000 *g* and the supernatant was extracted (C_{18} column). For total F_2-isoprostane (8-iso-prostaglandin $F_{2\alpha}$) determination, urine samples were hydrolysed by incubation at 40 °C for 90 min with 10 M NaOH. The samples were allowed to cool and were neutralized with 2 M HCl. After centrifugation, the supernatant was collected for assay. Total F_2-isoprostanes were measured by a competitive enzyme immunoassay (R&D Systems, Minneapolis, MN, U.S.A.). Plasma levels of MDA were assessed using the method described by Esterbauer and Cheeseman [21]. Plasma NOx was measured using nitrate reductase and Griess reaction [22]. Sodium, potassium, calcium, urea, creatinine and plasma protein were measured on the day using an autoanalyser (CX4; Beckman, Brea, CA, U.S.A.). Proteinuria was measured by the method of Bradford [23].

Histological techniques

For conventional morphology, buffered 4 % formaldehyde-fixed paraffin-embedded longitudinal tissue sections in the sagittal plane were stained with haematoxylin and eosin, Masson–Goldner trichromic stain and periodic acid Schiff (PAS) stain. The extent of vascular injury [stenosis, hyaline arteriopathy (HA) and myointimal hyperplasia (MH)] was assessed by examining profiles of arteries and arterioles in a single kidney section. The presence of glomerular lesions (glomerulosclerosis, collapse and capsular fibrosis) was evaluated in at least 200 glomeruli. Tubular atrophy, tubular casts and tubular dilation were also evaluated. The morphological study was done in blinded fashion on sections with light microscopy, using the most appropriate stain for each lesion. The values were expressed as the percentage of rats with lesions in each group, and the severity of lesions was calculated semi-quantitatively using a 0 to 3+ scale [−, absence; +, mild (< 10 % of vessel, tubules or glomeruli involved); ++, moderate (10–25 %); +++, severe (> 25 %)].

Statistical analyses

The evolution of tail SBP with time was compared using a nested design. When the overall difference was significant, Bonferroni's method with an appropriate error was used. The remaining variables measured at the end of the experimental period were compared with one-way ANOVA, and subsequent pair-wise comparisons with the Newmann–Keuls test.

RESULTS

BP

Figure 1 shows the evolution of the tail SBP measured by plethysmography (Figure 1, left-hand panel) and the final MAP measured by direct recording in conscious rats (Figure 1, right-hand panel). SBP rose in SHR-controls during the 9 weeks of the study. Figure 1 also shows that DOCA treatment aggravated the evolution

Table 1 Morphological variables measured at the end of the experimental period

Data are means ± S.E.M. $^*P < 0.05$ compared with the SHR-control group. $n = 8$ in all groups. LKW, left kidney weight.

	Group			
Variable	SHR-control	SHR + losartan	SHR + DOCA	SHR + DOCA + losartan
Final BW (g)	347 ± 9.5	342 ± 2.7	347 ± 7.9	363 ± 6.9
LKW (mg)	984.0 ± 14.0	975.1 ± 19.4	1045 ± 31.6*	1133 ± 38.1*
LVW (mg)	980.0 ± 11.2	823.9 ± 21.8*	1071 ± 32.8*	1051 ± 31.4
LKW/BW (mg/g)	2.83 ± 0.041	2.85 ± 0.050	3.02 ± 0.058*	3.11 ± 0.073*
LVW/BW (mg/g)	2.82 ± 0.030	2.41 ± 0.049*	3.10 ± 0.113*	2.89 ± 0.075
LVW/RVW (mg/g)	6.04 ± 0.27	4.89 ± 0.18*	7.28 ± 0.30*	6.81 ± 0.38*

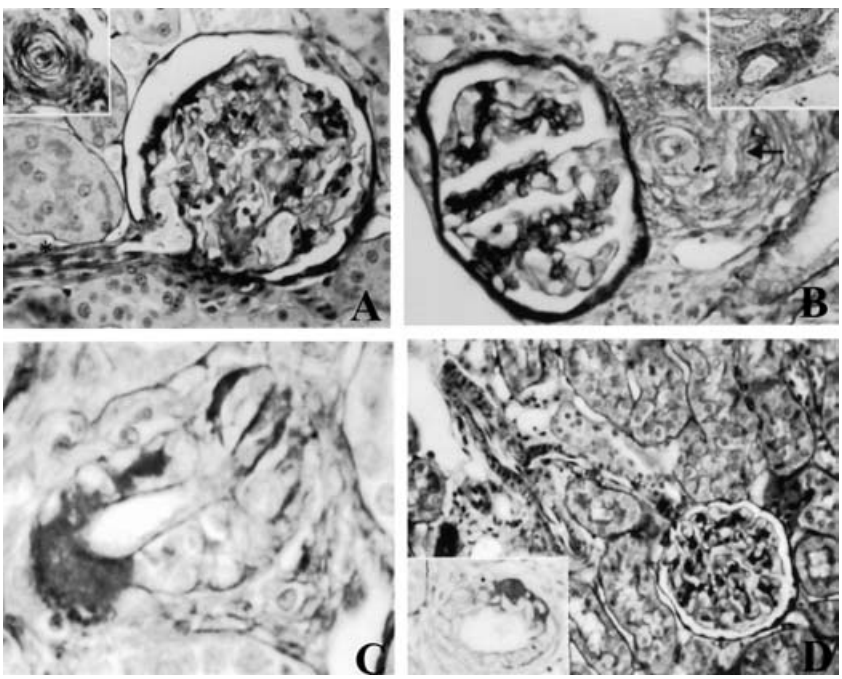

Figure 2 Renal lesions in SHR groups

(A) Vasoconstriction of afferent glomerular arteriole (*) with absence of glomerulosclerosis in the SHR-control group (stained with PAS; magnification, × 140). Inset: moderate/severe MH (stained with PAS stain; magnification, × 70). (B) Glomerulus with prominent capsular fibrosis and increased arteriolar MH (arrow) in the SHR + DOCA group (stained with PAS; magnification, × 140). Inset: hyaline deposits with mild decrease of vascular lumen (stained with PAS; magnification, × 70). (C) Moderate HA in the SHR-control group (stained with PAS; magnification, × 280). (D) Absence of glomerular injury in the SHR + DOCA + losartan group (stained with haematoxylin/eosin; magnification, × 70). Inset: decreased vascular HA (stained with PAS; magnification, × 70).

of spontaneous hypertension, whereas losartan produced a significant attenuation in controls (SHR + losartan) and the DOCA-treated group. These findings were confirmed by direct BP measurement from the arterial catheter in conscious rats at the end of the experiment.

Morphological variables

Final BW was similar in all of the experimental groups. Relative left kidney weight was increased in both DOCA-treated groups, and losartan did not significantly modify this variable in control or DOCA-treated rats. The administration of DOCA increased left ventricular weight (LVW) relative to BW and relative to right ventricular weight (RVW; LVW/RVW), which are both indexes of left ventricular hypertrophy. Losartan reduced both LVW/BW and LVW/RVW ratios in control rats, but did not significantly modify these variables in DOCA-treated rats (Table 1).

The lesions in this rat model were mild, with the exception of HA (Figure 2). Table 2 shows the semi-quantitative morphological evaluation. In the SHR-control group, HA was the main and most intense lesion, with a thickening of vascular wall and decrease of lumen in 75 % of the group, and a mild/moderate MH

Table 2 Morphological features of SHR rat kidney after treatments

Values are expressed as a percentage of rats with lesions in each group, with the intensity in parentheses (+, mild intensity of vascular, glomerular and tubular lesions; ++, moderate intensity; +++, severe intensity; −, no lesion). $n = 8$ in all groups.

	Group			
Morphological feature	SHR-control	SHR + losartan	SHR + DOCA	SHR + DOCA + losartan
Glomerulosclerosis	0 (−)	0 (−)	0 (−)	0 (−)
Glomerular necrosis	0 (−)	0 (−)	0 (−)	0 (−)
Capsular fibrosis	50 (+)	25 (+)	85.7 (+)	28.5 (+)
HA	75 (++)	12.5 (+)	71.4 (+++)	42.8 (++)
MH	25 (+/++)	12.5 (+)	57.1 (+++)	14.2 (+)
Luminal obliteration	12.5 (+)	0 (−)	42.8 (++)	12.5 (+)
Tubular dilation	0 (−)	0 (−)	57.1 (+)	0 (−)
Tubular atrophy	12.5 (+)	0 (−)	57.1 (+)	28.5 (+)
Cast	0 (−)	0 (−)	14.2 (+)	0 (−)

in 25 %. No glomerulosclerosis or glomerular necrosis was observed. Capsular fibrosis, an indicator of hypertension, was present in 50 % of the group, and the tubulointerstitial damage was very scant (12.5 %). The administration of DOCA aggravated renal injury; thus the SHR + DOCA group presented glomeruli with prominent capsular fibrosis (85.7 %) and, almost always in medium-sized vessels, severe (+++) HA (71.4 %) and MH (57.1 %). There was a greater incidence of mild tubular atrophy and of mild and scattered casts compared with the SHR-control group. Losartan reduced the intensity and incidence of HA and MH in both losartan-treated SHR groups. The SHR + losartan group had 12.5 % HA (+) and 12.5 % MH (+), and the SHR + DOCA + losartan group had 42.8 % HA (++) and 14.2 % MH (+). The SHR + losartan group had no glomerular or tubulointerstitial injuries in renal parenchyma.

Plasma and urinary variables

Plasma sodium, potassium and creatinine were similar in all groups (results not shown). Plasma NOx was unaffected by DOCA and was increased in the SHR + losartan group, but losartan treatment was unable to significantly increase plasma NOx in DOCA-treated rats (Figure 3). Plasma MDA was increased in DOCA-treated rats and reduced in both losartan-treated groups (Figure 3). Plasma Ang II levels were reduced in the SHR + DOCA group, and this variable was increased in both losartan-treated groups in comparison with their controls (Table 3).

No significant differences were observed in food and fluid intake between any experimental group and the control group (results not shown). Creatinine clearance, diuresis, natriuresis and kaliuresis were also similar between the control and experimental groups (results not shown). DOCA administration produced a non-significant increase in proteinuria (SHR-control, 1.79 ± 0.07; and SHR + DOCA, 2.18 ± 0.18 mg of protein/mg of creatine); treatment with losartan did not significantly modify this variable in SHRs (SHR + losartan, 1.59 ± 0.13 mg of protein/mg of creatine), whereas it significantly attenuated proteinuria in DOCA-treated rats (SHR + DOCA + losartan, 1.53 ± 0.13 mg of protein/mg of creatine; $P < 0.05$). The DOCA-treated groups showed increased levels of urinary F_2-isoprostanes, and losartan significantly reduced urinary F_2-isoprostanes in the SHR and SHR + DOCA groups (Figure 3). Total urinary ET was not significantly affected by DOCA, but was significantly reduced by losartan in the SHR and DOCA-treated groups (Table 3).

Tissue Ang II and ET levels

The SHR + DOCA group had similar levels of cortical and medullary Ang II compared with those observed in the SHR-control group, but had a lower concentration in the left ventricle (Table 3). Losartan reduced the levels of Ang II in renal cortex and left ventricle in SHRs and produced a reduction in Ang II levels in the renal cortex and medulla of DOCA-treated SHRs, with no change in the levels of this peptide in the left ventricle compared with SHRs not treated with DOCA. Ventricular ET concentration was similar in all groups. DOCA produced increased levels of ET in the renal cortex, and losartan produced significant reductions in renal (cortex and medulla) ET levels in both treated groups when compared with their respective controls (SHR-control or SHR + DOCA groups; Table 3).

DISCUSSION

BP

The present study shows that the course of hypertension in SHRs is aggravated by the administration of DOCA at a dose that does not increase BP in normal rats [18]. Although the mechanisms by which DOCA accelerates

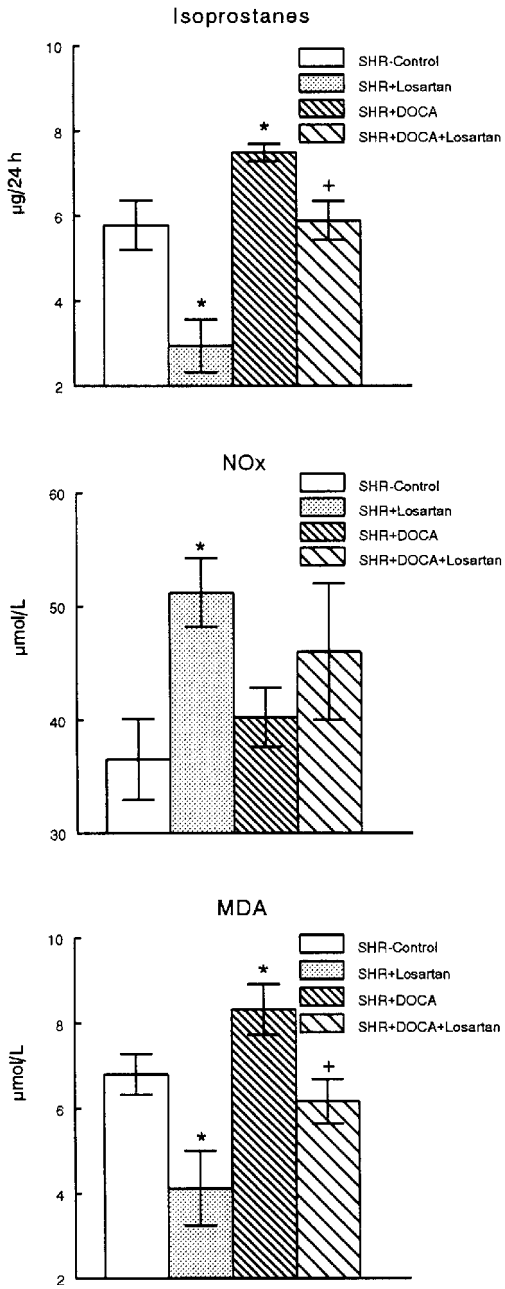

Figure 3 F_2**-isoprostanes in 24-h urine collection and plasma NOx and plasma MDA measured at the end of the experimental period**

Data are means ± S.E.M. $^*P < 0.05$ compared with the SHR-control group; $+ P < 0.05$ compared with the SHR + DOCA group.

spontaneous hypertension are not well defined, the present study shows that DOCA-treated SHRs have an increased 24-h urinary excretion of isoprostane $F_{2\alpha}$ and have increased levels of ET in the renal cortex as well as unsuppressed renal Ang II, which could contribute to elevate BP further. On the other hand, the sodium-retaining effects of mineralocorticoids may play a major role. DOCA might aggravate the displacement to the right of the pressure–diuresis–natriuresis curve in SHRs [24]. In fact, the SHR + DOCA group showed normal natriuresis with an increased BP, suggesting a defective pressure–natriuresis relationship.

Our present data also showed that losartan reduced BP in SHRs. However, one of the main observations of the present study was that losartan reduced BP in low-renin DOCA-treated SHRs. The fact that losartan reduces BP in both groups to a similar degree indicates that this phenomenon may be independent of the renin status, suggesting that genetic factors, but not mineralocorticoid components of BP, are reduced by losartan. These findings contrast with the suppression by DOCA of the antihypertensive effect of losartan in NO-inhibited hypertensive rats [18], and with the widely recognized inability of AT_1 receptor blockade to reduce BP in low-renin DOCA-salt hypertensive rats [19,20]. The mechanism by which losartan unexpectedly reduced BP in DOCA-treated SHRs is not clear, but attenuation of the increased oxidative stress and a reduction in the renal levels of ET and Ang II could participate in this phenomenon.

Oxidative stress

Our group [8] has reported previously that 24-h urinary isoprostane $F_{2\alpha}$ excretion and plasma MDA levels are increased in SHRs in comparison with Wistar–Kyoto rats, and the present results show that DOCA administration produced an increase in plasma levels of MDA and in the 24-h urinary excretion of isoprostane $F_{2\alpha}$, suggesting that mineralocorticoids aggravate oxidative stress in SHR. The present study also showed that long-term treatment with losartan reduced 24-h urinary excretion of isoprostane $F_{2\alpha}$ and plasma MDA levels, although it did not normalize these variables. Therefore, our findings indicate that chronic oral treatment with losartan reduces oxidative stress in the SHR, regardless of the renin status, which might contribute to its beneficial effects on BP and renal injury. However, losartan only improved plasma NOx in SHRs that were not treated with DOCA, suggesting that an unsuppressed renin is required for this effect. This is consistent with the well-known functional balance between Ang II and NO [25].

Cardiac hypertrophy

The hypotensive effect of losartan or angiotensin-converting-enzyme (ACE)-inhibitors in SHRs is associated with a reduction in cardiac hypertrophy [2,27]. It has even been observed that low doses of RAS blockers that do not reduce BP produce an important regression of cardiac hypertrophy [27]. Moreover, it has been reported [2] that RAS components in the heart are increased in SHRs and that the administration of losartan or captopril is followed by a decreased ventricular content of Ang II, which has been related to the regression of left ventricular hypertrophy [2].

Table 3 Ang II and ET levels measured in biological fluids and in cardiac and renal tissues at the end of the experimental period

Data expressed as means ± S.E.M. $^*P < 0.05$ compared with the SHR-control group; †$P < 0.05$ compared with the SHR-DOCA group. $n = 8$ in all groups.

	Group			
	SHR-control	SHR + losartan	SHR + DOCA	SHR + DOCA + losartan
Ang II				
Plasma (pg/ml)	48.51 ± 3.35	98.17 ± 7.28*	19.71 ± 1.44*	28.37 ± 5.47*†
Tissues				
Cortex (pg/g)	439.1 ± 22.5	316.5 ± 22.6*	410.6 ± 34.5	324.6 ± 13.7*†
Medulla (pg/g)	349.3 ± 21.2	394.1 ± 19.5	315.5 ± 17.6	226.1 ± 14.8*†
Left ventricle (pg/g)	157.6 ± 10.0	135.9 ± 5.0*	117.5 ± 12.4*	124.0 ± 9.41*
ET				
Urine (pg/24 h)	669.2 ± 68.9	395.2 ± 26.6*	721.1 ± 40.2	439.0 ± 33.9*†
Tissues				
Cortex (pg/g)	193.1 ± 24.5	132.3 ± 11.5*	375.7 ± 40.1*	263.8 ± 32.8*†
Medulla (pg/g)	1497 ± 191	1086 ± 77*	1479 ± 209	357 ± 58*†
Left ventricle (pg/g)	64.09 ± 4.68	72.26 ± 4.63	68.53 ± 6.85	82.89 ± 8.50

All of the above observations indicate that the RAS, especially the cardiac RAS, plays an important role in the cardiac hypertrophy of SHRs. Our present data show that losartan reduced ventricular hypertrophy and ventricular Ang II in SHRs, confirming previous observations in SHRs [2]. However, DOCA-treated rats showed a greater hypertrophy with reduced ventricular levels of Ang II, and losartan was unable to significantly decrease ventricular hypertrophy and ventricular Ang II in DOCA-treated rats, despite producing an important reduction in BP. These results indicate that Ang II levels might determine the efficacy of AT_1 receptor blockade to prevent cardiac hypertrophy in hypertension. Moreover, these results suggest that ventricular Ang II does not participate in the increased ventricular hypertrophy induced by DOCA administration in which a role may be played by the greater BP of DOCA-treated rats, the sympathetic tone [4] and/or the direct hypertrophic and fibrotic effect of mineralocorticoids [28].

Several observations suggested that ET, which is known to have powerful hypertrophic and mitogenic properties [29], might contribute to cardiac hypertrophy in SHRs and low-renin SHR + DOCA. However, the data reported in the present study showed that ventricular ET was not significantly modified by DOCA or losartan treatment, suggesting that ventricular ET does not plays a role in the increased and decreased cardiac hypertrophy induced by DOCA and losartan treatment respectively, in SHRs.

Renal injury and proteinuria

The 19-week-old SHRs in the present study showed a mild proteinuria compared with normotensive rats in our laboratory [18,30], and our examination of the renal tissue showed no major renal histopathological changes. The SHR model is characterized by an increased BP, but the kidney is protected against renal injury by an increased resistance in the afferent arteriole [31]. Proteinuria starts to increase at 5 months of age and rapidly accelerates after 10 months of age [32]. Because the rats were evaluated at 4 months of age in our present study, no markedly increased proteinuria was recorded. The same explanation holds true for the absence of a marked glomerulosclerosis. DOCA aggravated proteinuria and renal injury and losartan was able to attenuate renal injury in SHRs and both alterations in DOCA-treated animals, as reported by Kim et al. [20] in DOCA-salt hypertensive rats.

Renal Ang II and ET

In the last few years, evidence has been obtained for hypertensinogenic and pathological actions of Ang II in the kidney [5,20]. According to the present results, losartan produces a reduction in the renal levels of Ang II that may play a role in the antihypertensive effects and renal protection of losartan in SHR and SHR + DOCA groups.

DOCA suppressed plasma Ang II, but did not reduce cortical and medullary renal Ang II, which might contribute to aggravate renal injury. One important feature in the RAS profile observed in these rats is that renal Ang II levels were greater than can be explained on the basis of circulating Ang II and suppressed renin activity [18]. These data are consistent with previous observations that suggested that angiotensin peptides continue to be generated intrarenally via a renin-independent pathway or that circulating Ang II accumulates within the kidney by internalization via an

AT_1 receptor-mediated process [33]. In fact, as reported above, losartan produced a reduction in renal Ang II in the SHR and SHR + DOCA groups in our present study.

ET plays a role in renal haemodynamic and excretory functions [34] and it has been reported [6] that enalapril decreased plasma ET levels in SHRs. In the present study, the highest concentration of ET was found in the renal medulla, as reported previously [35]. DOCA increased ET in the renal cortex and losartan administration produced a significant reduction in renal and urinary levels of ET in the SHR and SHR + DOCA groups. The source of urinary ET is not entirely established, but is likely to be of renal origin [36], because its clearance from plasma is very low and tiny levels of ET are usually found in plasma in hypertension, whereas relatively high amounts are excreted in the urine [37]. In summary, these results suggest that the increased renal levels of ET might participate in the accelerated renal injury induced by DOCA administration, and that losartan produces a reduction in renal levels of Ang II and ET, unrelated to the renin status of the animal in the case of the SHR, which might play a role in the renoprotective effect of this drug.

Conclusions

The results of the present study show that DOCA administration aggravates the course of spontaneous hypertension, increasing BP, ventricular hypertrophy and renal injury. DOCA-treated SHRs have augmented oxidative stress and increased levels of ET in the renal cortex, which could contribute to aggravate hypertension. Losartan treatment reduced oxidative stress, renal Ang II and ET and urinary ET in SHR and SHR + DOCA groups, regardless of their renin status, which might contribute to the beneficial effects of this drug on BP and renal injury.

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