Differential evolution of blood pressure and renal lesions after RAS blockade in Lyon hypertensive rats

DELPHINE BERTRAM,1 NELLY BLANC-BRUNAT,2 JEAN SASSARD,1 AND MING LO1

1Département de Physiologie et Pharmacologie Clinique, Centre National de la Recherche Scientifique Unité Mixte de Recherche 5014, Institut Fédératif de Recherche 39, Faculté de Pharmacie, 69373 Lyon Cedex 08; and 2Institut National de la Santé et de la Recherche Médicale Unité 80, Hôpital Edouard Herriot, 69437 Lyon Cedex 03, France

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Bertram, Delphine, Nelly Blanc-Brunat, Jean Sassard, and Ming Lo. Differential evolution of blood pressure and renal lesions after RAS blockade in Lyon hypertensive rats. Am J Physiol Regul Integr Comp Physiol 283: R1041–R1045, 2002.—The present work aimed to assess, in Lyon hypertensive (LH) rats, whether an early and prolonged inhibition of the renin-angiotensin system (RAS) could result in a blood pressure (BP) lowering and nephroprotection that persist after its withdrawal. Male LH rats received orally from 3 to 12 wk of age either an angiotensin-converting enzyme inhibitor perindopril at the doses of 0.4 and 3 mg·kg⁻¹·day⁻¹ or an AT₁ receptor antagonist losartan at the dose of 10 mg·kg⁻¹·day⁻¹. BP, histological changes in the kidney, and urinary protein excretion were examined during and 10 wk after cessation of the treatments. Both perindopril and losartan decreased BP, prevented renal lesions, and limited urinary protein excretion. After cessation of the treatment, BP returned to the level of never-treated LH rats in rats having received 3 mg·kg⁻¹·day⁻¹ of perindopril while it remained slightly lower in those treated with 0.4 mg·kg⁻¹·day⁻¹ of perindopril or with losartan. This lack of marked persistent antihypertensive effect contrasted with a durable decrease in urinary protein excretion and improvement of the renal histological lesions. In conclusion, it is possible to separate the BP-lowering effects of RAS blockade from those on glomerulosclerosis and urinary protein excretion.

angiotensin-converting enzyme; AT₁ receptor antagonist; glomerulosclerosis

In LYON GENETICALLY HYPERTENSIVE (LH) rats, as well as in spontaneously hypertensive rats (SHR), a blockade of the renin-angiotensin system (RAS) fully prevents the development of hypertension and much of the accompanying target-organ damages (15, 16). Interestingly, several studies showed that, in young SHR, an early and short-term blockade of RAS induced decreases in blood pressure (BP) and improvements in vascular and renal function that persisted after treatment withdrawal (4, 6, 8, 13, 24). When the blockade was induced in adult SHR, such persistent effects were either modest or absent (13, 20, 26). In addition, it is noteworthy that the long-lasting BP decrease observed after blockade seems specific of the RAS blockade, as it is not observed with vasodilators, β-blockers, or calcium antagonists (4, 8, 24). A likely explanation is that an early and brief increase in RAS activity may be sufficient to allow the development of a stable hypertension. Such an hypothesis is favored by the observation that plasma renin is increased in young hypertensive rats and thereafter reduced in adult animals (23, 27). However, the observation that in LH rats captopril given from conception to weaning was devoid of persistent effects on BP after its cessation (7) argues against this hypothesis. Nevertheless, because in LH rats the RAS was never blocked during the period of fast rise of BP (4 to 10 wk of age), we thought it of interest to determine whether a blockade of the RAS using an angiotensin-converting enzyme (ACE) inhibitor or an AT₁ receptor antagonist during this period might induce a BP decrease and a renoprotection that persist after treatment withdrawal.

MATERIALS AND METHODS

Animals. Fifty male LH rats (28) were used. They were housed two to three per cage under controlled conditions (temperature: 21 ± 1°C; humidity: 60 ± 10%; lighting: 8–20 h) and fed a standard rat chow containing 0.3% sodium (Elevage UAR A03, Villemoisson-sur-Orge, France) and tap water ad libitum. The studies were conducted in accordance with our institutional guidelines for animal care.

Experimental protocols. At 3 wk of age, LH rats were randomly divided into four groups. Twelve LH rats remained untreated and served as controls; the others were treated orally with losartan (LH-lo; 10 mg·kg⁻¹·day⁻¹) and fed a standard rat chow containing 0.3% sodium (Elevage UAR A03, Villemoisson-sur-Orge, France) and tap water ad libitum. The studies were conducted in accordance with our institutional guidelines for animal care.
nary sodium concentration was measured by flame photometry (model 243, ILmeter, Lexington, MA) and urinary protein excretion by a colorimetric method (29). Then, the right kidney was removed in half of the rats of each group under halothane (2% in oxygen) anesthesia to examine the renal histology. The treatments were started at the age of 13 wk. At 21 wk of age, 24-h urines were collected to measure the urinary sodium and protein excretion. Between 10 and 21 wk of age, indirect systolic BP (SBP) was measured each 2 wk (between 9 and 13 h) by tail-cuff plethysmography (Narco Biosystem, Houston, TX) in conscious preheated (37°C for 10–15 min) animals. At 22 wk of age, intra-aortic BP was recorded in freely moving rats through a polyethylene catheter (PE-10 fused to PE-50) inserted while rats were under anesthesia with halothane (2% in oxygen) via the left femoral artery in the abdominal aorta. The catheter was filled with heparinized saline (25 IU/ml), guided subcutaneously, and exteriorized at the back of the neck. After a 2-day recovery, the arterial catheter was connected to a pressure transducer (Statham P23 ID, Gould, Cleveland, OH) via a rotating swivel that allowed the animal to move freely. Recordings began 1 h after connection to the transducer. With the use of our computerized technique (10), 2-h aortic BP curve (between 10 and 12 h) was digitized and processed on-line by a computer (MVME SYS121, Motorola, Tempe, AZ) to determine and store beat-to-beat values of SBP and diastolic BP (DBP) as well as of heart rate. Then, all rats were euthanized with pentobarbital sodium, and the kidneys were dissected out for histological analysis.

**Histological analysis of the kidneys.** After removal, kidneys were halved, hemisections fixed in Bouin’s solution, and then embedded in paraffin. Standard stainings were applied to 2.5-μm-thick slices: hematoxylin-eosin, periodic-acid Schiff reagent, Masson’s trichrome, silver methenamine, and modified May-Grundwald Giemsa stain. Semiquantitative evaluation for glomerular, vascular, tubular, and interstitial lesions was performed in a blinded fashion. The importance of the lesions in each animal was graded from 0 to 3 (0 = normal; 0.5 = minimal; 1 = slight; 2 = moderate; and 3 = severe). The mean of the grades was used to characterize each group of animals.

**Statistical analysis.** Data are expressed as means ± SE. Comparisons between groups used one-way ANOVA with treatment as factor followed by a Fisher test. Comparison of indirect SBP evolution with age used two-way ANOVA with repeated measures over time. *P < 0.05* was considered as significant.

**RESULTS**

**Effects of RAS blockade.** After 9 wk of treatment, i.e., in 12-wk-old rats, losartan (LH-lo) and the low dose of perindopril (LH-lp) induced similar decreases in SBP and in urinary protein excretion (Table 1). LH-lp, but not LH-lo, rats exhibited a lower body weight than untreated LH rats and a larger diuresis than both untreated and losartan-treated LH rats. The high dose of perindopril (LH-hp) induced a more marked antihypertensive effect associated with a further decrease in urinary protein excretion and a significant increase in both diuresis and natriuresis. When considering the four groups of rats, the urinary protein excretion was closely related to the SBP level (*r* = 0.705; *n* = 50; *P* < 0.001).

**BP evolution after treatment withdrawal.** Figure 1 shows that SBP rapidly increased after treatment withdrawal to reach values close to those of untreated LH rats. Between 13 and 21 wk of age, SBP remained slightly lower in LH-lo and LH-lp than in never-treated LH rats (2-way ANOVA, *P* < 0.05 for both groups), whereas the SBP of LH-hp rats exhibited a tendency to overreach that of untreated LH rats. Intra-aortic BP measurements performed in 22-wk-old rats (Table 2) showed that 10 wk after cessation of the treatments, losartan and the low dose of perindopril did not have persistent effects on BP, diuresis, and natriuresis. However, in both groups, the urinary protein excretion remained significantly lower than in untreated LH rats. In LH rats having received the high dose of perindopril, 10 wk after treatment withdrawal, SBP was higher than that of the LH-lo and LH-lp rats, and the diuresis was larger than that of untreated LH rats. However, the urinary protein excretion remained decreased despite the redevelopment of hypertension.

**Table 1. Body wt, indirect SBP, urinary protein excretion, diuresis, and natriuresis in 12-wk-old LH rats in which the renin-angiotensin system was blocked since weaning**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Wt, g</th>
<th>SBP, mmHg</th>
<th>UprotV, mg/24 h</th>
<th>UV, ml/100 g·24 h</th>
<th>UNaV, mmol/100 g·24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>12</td>
<td>353 ± 8</td>
<td>152 ± 3</td>
<td>85 ± 7</td>
<td>3.0 ± 0.1</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>LH-lo</td>
<td>13</td>
<td>340 ± 5</td>
<td>129 ± 2</td>
<td>42 ± 3*</td>
<td>3.2 ± 0.2</td>
<td>0.55 ± 0.03</td>
</tr>
<tr>
<td>LH-lp</td>
<td>13</td>
<td>327 ± 7*</td>
<td>122 ± 2*</td>
<td>41 ± 2*</td>
<td>4.5 ± 0.3*</td>
<td>0.55 ± 0.02</td>
</tr>
<tr>
<td>LH-hp</td>
<td>12</td>
<td>318 ± 8†‡</td>
<td>97 ± 3†‡</td>
<td>27 ± 1†‡</td>
<td>6.2 ± 0.2*†‡</td>
<td>0.63 ± 0.03*†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. LH, untreated Lyon hypertensive; LH-lo, losartan-treated LH (10 mg·kg⁻¹·day⁻¹); LH-lp, low dose of perindopril-treated LH (0.4 mg·kg⁻¹·day⁻¹); LH-hp, high dose of perindopril-treated LH rats (3 mg·kg⁻¹·day⁻¹); SBP, systolic blood pressure; UprotV, urinary protein excretion; UV, diuresis; UNaV, natriuresis. *P < 0.05 LH; †*P < 0.05. LH-lo; and ‡P < 0.05. LH-hp.
At that stage using the four groups of rats, the urinary protein excretion was no longer correlated to the SBP level ($r = 0.041$; $n = 28$; not significant).

Renal lesions. As shown in Fig. 2, A–D, and summarized in Table 3, histological analysis in 12-wk-old young LH rats confirmed the existence of the renal lesions previously described in LH rats (2): focal glomerulosclerosis with thickening of the capsular basement membrane, arteriolar hypertrophy on the initial stage, and slight tubular dilatation. Losartan and perindopril fully prevented the segmental glomerulosclerosis and arteriolar hypertrophy. However, only losartan and the low dose of perindopril decreased significantly the tubule dilatation.

In 22-wk-old untreated LH rats, the renal lesions increased (Fig. 2 and Table 3), i.e., segmental glomerulosclerosis with fibrosis of capsule and hyaline droplets, arteriolar hypertrophy, dilated tubules with flattened epithelia, hyaline casts in tubular lumen, and interstitial inflammatory infiltration with sclerofibrosis (Fig. 2E). In 22-wk-old LH rats treated between 3 and 12 wk of age, glomerulosclerosis remained minimal or absent in all the rats 10 wk after treatment withdrawal (Fig. 2, F–H). Losartan and the low dose of perindopril induced a persistent amelioration in arteriolar hypertrophy. It is noteworthy that, in terms of histological lesions, the high dose of perindopril was not more efficient than the low one.

DISCUSSION

The present work demonstrates that, despite a full prevention of the development of hypertension in LH rats by perindopril or losartan, the effects of RAS...
blockade on BP did not persist after cessation of the treatments, whereas, on the contrary, the renoprotection appeared to be longer lasting.

The development of hypertension in LH rats is characterized by two stages, i.e., first a rapid rise in BP from 4 to 10 wk of age, and then a slower elevation. The efficacy of pharmacological blockade of the RAS in LH rats has been previously observed at any stage (15–17), thus suggesting that, despite the low renin secretion seen in adult LH rats (1, 27), high BP in this strain depends on an active RAS. It was repeatedly observed in SHR that the BP decrease induced by RAS blockade persisted for long periods of time after cessation of the treatment, provided that the latter was given in young animals (4, 8, 13, 24). These observations prompted us to test the hypothesis that an early and short-lasting increase in RAS activity could be sufficient to allow for the development of a life-long hypertension in LH rats. Following a protocol used in SHR (31), we previously observed that captorpril given from conception to weaning did not exhibit persistent effects after its cessation in LH rats (7). However, it remained possible that the RAS blockade, which in that experiment was stopped at 3 wk of age, did not totally cover the critical phase (between 4 and 10 wk of age) during which the RAS may be crucial for the development of hypertension in LH rats. In the present work using an ACE inhibitor or an AT1 receptor antagonist, we measured the effects of a RAS blockade performed during the period of fast rise in BP and also examined its long-term consequences on BP, urinary protein excretion, and renal histological lesions.

The treatments used markedly decreased the BP of LH rats and fully prevented the development of glomerulosclerosis while they lowered the urinary excretion of proteins. However, BP of LH rats started to rise soon after cessation of RAS blockade so as to reach or even overreach the level of never-treated, age-matched LH rats. Our observations are unlikely to be related to the compounds used, because in SHR, marked persistent effects on BP of RAS blockade were reported using several drugs, including perindopril at the doses of 0.4 (3) and 3 mg·kg⁻¹·day⁻¹ (12, 13) that we used here. The majority of SHR studies have been done using an indirect tail-cuff method. However, this persistent effect on BP was also observed when BP was measured via an arterial catheter in conscious SHR (4, 13) or by using a radiotelemetry system (14). The mechanisms underlying the persistence of the effects of RAS blockade in SHR remain unknown. The most frequent hypotheses involved the stimulatory effects of ANG II on various growth factors in the vessels and/or in the kidneys. As a consequence of an early and prolonged RAS blockade, the decrease in growth factors may have induced a long-lasting normalization of vascular structure and reactivity (8, 13, 22, 24). In the kidneys, the key organ in the long-term regulation of fluid volume and BP (9, 11), this early treatment prevented the development of renal lesions (21, 32) and ameliorated the renal function (5, 6). In previous studies (15, 16), we observed that chronic blockade of the RAS from 3 wk to adult age in LH rats prevented the development of hypertension, normalized the regional flows, and ameliorated the renal function.

After cessation of the treatment, the use of LH rats allowed us to clearly differentiate the long-term effects of RAS blockade on BP from those on glomerulosclerosis and protein excretion. This suggests that the period and/or pathways linking ANG II to glomerulosclerosis and urinary protein excretion may differ from those involved in the BP control. Because glomerulosclerosis requires a growth factor-dependent increase in the extracellular matrix formation (19, 21, 30), it is possible that an early and prolonged blockade of the RAS would stop this process. Finally, the present work shows that, at least in terms of glomerulosclerosis and urinary protein excretion, the highest dose of ACE inhibitor used is not more efficient than a lower, less antihypertensive dose. This finding is in close agreement with two other observations, one made in SHR (25) and one in Milan hypertensive rats (18).

In conclusion, the present work demonstrates the lack of marked antihypertensive effect after RAS blockade withdrawal in LH rats. In addition and more importantly, it shows that a short-term and early ACE inhibition or AT1 receptor antagonism can protect from glomerulosclerosis and proteinuria on the long term.

### REFERENCES


