Differential evolution of blood pressure and renal lesions after renin-angiotensin system blockade in Lyon hypertensive rats

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Short title: Persistent effects of RAS blockade

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Abstract

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 nephro-protection which persist after its withdrawal. Male LH rats received orally from 3 to 12 weeks of age, either an angiotensin converting enzyme (ACE) inhibitor, perindopril, at the doses of 0.4 and 3 mg/kg/day, or an AT1 receptor antagonist, losartan, at the dose of 10 mg/kg/day. BP, histological changes in the kidney and urinary protein excretion were examined during and 10 weeks 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 the 3 mg/kg/day of perindopril while it remained slightly lower in those treated with 0.4 mg/kg/day 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.

Key words: blood pressure, angiotensin converting enzyme, AT1 receptor antagonist, glomerulosclerosis


**Introduction**

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 which persisted after treatment withdrawal (3, 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 (3, 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, since in LH rats, the RAS was never blocked during the period of fast rise of BP (4 to 10 weeks of age), we thought it of interest to determine whether a blockade of the RAS using an ACE inhibitor or an AT1 receptor antagonist during this period might induce a BP decrease and a reno-protection which persist after treatment withdrawal.
Material and Methods

Animals

Fifty male LH rats (28) were used. They were housed 2-3 per cage under controlled conditions (temperature: 21 ± 1°C; humidity: 60 ± 10%; lighting: 8-20h) 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 weeks 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/day, n = 13, Du-Pont Merck Pharmaceuticals Co., St. Louis, Missouri, USA) an AT1 receptor antagonist or with perindopril (Servier Laboratories, Neuilly-sur-Seine, France) an ACE inhibitor at a low dose (LH-lp, 0.4 mg/kg/day, n = 13) or at a high dose (LH-hp, 3 mg/kg/day, n = 12). Drugs were given in drinking water and their concentration adjusted weekly according to body weight and water intake. At 12 weeks of age, the animals were placed in individual metabolic cages. After a 2-day habituation period, 24-hour urines were collected. Urinary sodium concentration was measured by flame photometry (model 243, ILmeter, Lexington, Massachusetts, USA) 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 in order to examine the renal histology. The treatments were stopped at the age of 13 weeks. At 21 weeks of age, 24-hour urines were collected to measure the urinary sodium and protein excretion. Between 10 and 21
weeks of age, indirect SBP was measured each two weeks (between 9 and 13h) by tail-cuff plethysmography (Narco Biosystem, Houston, Texas, USA) in conscious preheated (37°C for 10-15 minutes) animals. At 22 weeks of age, intra-aortic BP was recorded in freely moving rats through a polyethylene catheter (PE10 fused to PE50) inserted 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, Ohio, USA) via a rotating swivel that allowed the animal to move freely. Recordings began 1h after connection to the transducer. Using our computerized technique (10), 2-hour aortic BP curve (between 10 and 12h) was digitized and processed on-line by a computer (MVME SYS121, Motorola, Tempe, Arizona, USA) to determine and store beat-to-beat values of systolic (SBP) and diastolic BP (DBP), as well as of heart rate (HR). Then all rats were euthanized with pentobarbital sodium, and the kidneys dissected out for histological analysis.

**Histological analysis of the kidneys**

After removal, kidneys were halved, hemisections fixed in Bouin’s solution then embedded in paraffin. Standard stainings were applied to 2.5µ thick slices: hematoxylin-eosin, periodic-acid Schiff reagent, Masson’s trichrome, silver methenamine and modified May-Grundwald Giemsa stain. Semi-quantitative 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 analysis of variance with treatment as factor followed by a Fisher test. Comparison of indirect SBP evolution with age used two-way analysis of variance with repeated measures over time. $P < 0.05$ was considered as significant.
Results

**Effects of renin-angiotensin system blockade (see Table 1)**

After 9 weeks of treatment, *i.e.*, in 12 week-old rats, losartan (LH-lo) and the low dose of perindopril (LH-lp) induced similar decreases in SBP and in urinary protein excretion. 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 4 groups of rats, the urinary protein excretion was closely related to the SBP level \( r = 0.705; n = 50; P < 0.001 \).

**Blood pressure evolution after treatment withdrawal**

Figure 1 shows that SBP rapidly increased after treatment withdrawal to reach values close to those of never treated LH rats. Between 13 and 21 weeks of age, SBP remained slightly lower in LH-lo and LH-lp than in never treated LH rats (two-way ANOVA, \( P < 0.05 \) for both groups) while the SBP of LH-hp rats exhibited a tendency to overreach that of untreated LH rats. Intra-aortic BP measurements performed in 22 week-old rats (Table 2) showed that 10 weeks 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 weeks after treatment withdrawal SBP was higher than that of the LH-lo and LH-lp rats, and the diuresis larger than that of untreated LH rats. However,
the urinary protein excretion remained decreased in spite of the redevelopment of hypertension. At that stage using the 4 groups of rats, the urinary protein excretion was no longer correlated to the SBP level ($r = 0.041; n = 28; n.s.$).

*Renal lesions*

As shown in Figure 2 (A, B, C, D) and summarized in Table 3, the histological analysis in 12 week-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 dilation.

In 22 week-old untreated LH rats, the renal lesions increased (Figure 2 and Table 3), *i.e.*, segmental glomerulosclerosis with fibrosis of capsule and hyaline droplets, arteriolar hypertrophy, dilated tubules with flattened epithelia and hyaline casts in tubular lumen, and interstitial inflammatory infiltration with sclero-fibrosis (Figure 2E). In 22 week-old LH treated between 3 and 12 weeks of age, glomerulosclerosis remained minimal or absent in all the rats ten weeks after treatment withdrawal (Figure 2F, G, 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, while, on the contrary, the reno-protection 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 weeks of age, then a slower elevation. The efficacy of pharmacological blockade of the RAS in LH rats has been previously observed at any stage (15, 16, 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 this latter was given in young animals (3, 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 have previously observed that captopril 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 weeks of age, did not totally cover the critical phase (between 4 and 10 weeks 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 examined also 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 (4) and 3 mg/kg/day (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 (3, 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 angiotensin 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, 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 weeks 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 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 angiotensin II to glomerulosclerosis and urinary protein excretion may differ from those involved in the BP control. Since 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


21. **Otsuda F, Yamauchi T, Kataoka H, Mimura Y, Ogura T, and Makino H.** Effects of chronic inhibition of ACE and AT1 receptors on glomerular injury in


Legends

Figure 1: Time course of indirect systolic blood pressure (SBP) in LH rats either untreated (LH) or treated with losartan (LH-lo), a low (LH-lp) or a high dose (LH-hp) of perindopril.

Figure 2: (x 400) A: in 12 week-old untreated LH rats, focal glomerulosclerosis (S) with thickening of the capsular basement membrane (F) and slight tubular dilatation. In 12 week-old LH rats treated with losartan (B), a low (C) or a high dose (D) of perindopril from 3 weeks of age, the reno-protection is observed in all treated animals. E: in 22 week-old never treated LH rats, segmental glomerulosclerosis (S) with increase of mesangial matrix (M), thickening of capillary loops, enlarged Bowman’s space, and fibrosis of the capsular basement membrane (F), dilated tubules, interstitial sclero-oedema and monocyte infiltration (arrow). In 22 week-old LH rats pre-treated with losartan (F), a low (G) or a high dose (H) of perindopril from 3 to 12 weeks of age, ten weeks after treatment withdrawal, the reno-protection persists in all animals.
Table 1. Body weight, indirect systolic blood pressure, urinary protein excretion, diuresis and natriuresis in 12 week-old LH rats in which the renin-angiotensin system was blocked since weaning.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW (g)</th>
<th>SBP (mmHg)</th>
<th>UprotV (mg/24h)</th>
<th>UV (ml/100g/24h)</th>
<th>UNaV (mmol/100g/24h)</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>
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<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>

Mean ± SE. LH, untreated LH; LH-lo, losartan-treated LH (10 mg/kg/day); LH-lp, low dose of perindopril-treated LH (0.4 mg/kg/day); LH-hp, high dose of perindopril-treated LH rats (3 mg/kg/day); BW, body weight; SBP, systolic blood pressure; UprotV, urinary protein excretion; UV, diuresis; UNaV, natriuresis. * P < 0.05 vs. LH; † P < 0.05 vs. LH-lo and ‡ P < 0.05 vs. LH-lp.
Table 2. Body weight, aortic systolic and diastolic blood pressure, urinary protein excretion, diuresis and natriuresis measured between 21 and 22 weeks of age, i.e., ten weeks after treatment cessation.

<table>
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<tr>
<th></th>
<th>( n )</th>
<th>( \text{BW} ) (g)</th>
<th>( \text{SBP} ) (mmHg)</th>
<th>( \text{DBP} ) (mmHg)</th>
<th>( \text{UprotV} ) (mg/24h)</th>
<th>( \text{UV} ) (ml/100g/24h)</th>
<th>( \text{UNaV} ) (mmol/100g/24h)</th>
</tr>
</thead>
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<tr>
<td>LH</td>
<td>6</td>
<td>433 ± 7</td>
<td>175 ± 1</td>
<td>116 ± 4</td>
<td>241 ± 26</td>
<td>2.6 ± 0.2</td>
<td>0.40 ± 0.02</td>
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<tr>
<td>LH-lo</td>
<td>5</td>
<td>432 ± 11</td>
<td>166 ± 3</td>
<td>111 ± 3</td>
<td>159 ± 20 *</td>
<td>3.2 ± 0.3</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>LH-lp</td>
<td>6</td>
<td>428 ± 6</td>
<td>167 ± 2</td>
<td>110 ± 3</td>
<td>153 ± 22 *</td>
<td>3.0 ± 0.1</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>LH-hp</td>
<td>6</td>
<td>436 ± 8</td>
<td>186 ± 8 †‡</td>
<td>121 ± 4 †‡</td>
<td>85 ± 8 *†‡</td>
<td>3.5 ± 0.5 *</td>
<td>0.50 ± 0.12</td>
</tr>
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</table>

Mean ± SE. LH, untreated LH; LH-lo, losartan-treated LH (10 mg/kg/day); LH-lp, low dose of perindopril-treated LH (0.4 mg/kg/day); LH-hp, high dose of perindopril-treated LH rats (3 mg/kg/day); BW, body weight; SBP and DBP, systolic and diastolic blood pressure; UprotV, urinary protein excretion; UV, diuresis; UNaV, natriuresis. Seven animals in each groups for urinary parameters (UprotV, UV and UNaV). * \( P < 0.05 \) vs. LH; † \( P < 0.05 \) vs. LH-lo and ‡ \( P < 0.05 \) vs. LH-lp.
Table 3. Histological lesion grade in the kidneys of 12 (during treatment) and 22 week-old (10 weeks after treatment cessation) LH rats.

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>Glomerulosclerosis</th>
<th>Arteriolar hypertrophy</th>
<th>Tubule dilation</th>
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</thead>
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<tr>
<td><strong>12 weeks:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>5</td>
<td>1.2 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>LH-lo</td>
<td>6</td>
<td>0 ± 0 *</td>
<td>0 ± 0 *</td>
<td>0.3 ± 0.2 *</td>
</tr>
<tr>
<td>LH-lp</td>
<td>6</td>
<td>0 ± 0 *</td>
<td>0 ± 0 *</td>
<td>0.5 ± 0.2 *</td>
</tr>
<tr>
<td>LH-hp</td>
<td>5</td>
<td>0 ± 0 *</td>
<td>0 ± 0 *</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td><strong>22 weeks:</strong></td>
<td>3</td>
<td>2.7 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>LH</td>
<td>4</td>
<td>0.3 ± 0.1 *</td>
<td>0.3 ± 0.3 *</td>
<td>0.9 ± 0.4 *</td>
</tr>
<tr>
<td>LH-lp</td>
<td>3</td>
<td>0.5 ± 0.2 *</td>
<td>0.3 ± 0.3 *</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td>LH-hp</td>
<td>5</td>
<td>0 ± 0 *</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

Mean ± SE (importance of the lesions was graded from 0 to 3). LH, untreated LH; LH-lo, losartan-treated LH (10 mg/kg/day); LH-lp, low dose of perindopril-treated LH (0.4 mg/kg/day); LH-hp, high dose of perindopril-treated LH rats (3 mg/kg/day). * $P < 0.05$ vs. LH at the same age.
Figure 1

SBP (mmHg) vs. Age (weeks) for different treatment groups:
- LH (n = 7)
- LH-lo (n = 7)
- LH-lp (n = 7)
- LH-hp (n = 7)
Figure 2

12w

A

B

C

D

22w

E

F

G

H

C

Los

P0.4

P3