Renin-Angiotensin System in Two Genetically Normotensive Strains of Lyon Rats

Pierre Lantelme, Ming Lo, and Jean Sassard

Compared to the Lyon normotensive (LN) controls, adult Lyon hypertensive rats (LH) exhibit a renin-angiotensin system (RAS) dependent hypertension despite a low renin secretion. This discrepancy could be explained by the elevated slow pressor response to angiotensin II (AII) found in LH rats compared to LN controls. To evaluate more precisely the pathophysiological importance of this increased response, the present work aimed at determining whether the characteristics of the RAS were identical in LN and low blood pressure (LL) rats, the other normotensive control strain simultaneously selected with LH rats. Plasma and kidney renin and prorenin were measured in 11-week-old LN and LL rats. Aortic blood pressure (BP) was recorded at 15 weeks of age in freely moving rats of both strains either untreated or having received an angiotensin converting enzyme inhibitor, perindopril (3 mg/kg/day orally) since the age of 3 weeks. Acute dose-response curves were constructed for AII and norepinephrine (NE). The long-term pressor effects of AII (200 ng/kg/min) and NE (1000 ng/kg/min) were measured after chronic infusions in perindopril-treated LN and LL rats. LN and LL rats exhibited similar mean BP level before (114 ± 2 and 117 ± 2 mm Hg, respectively) and after perindopril treatment (91 ± 3 and 93 ± 1 mm Hg, respectively). Plasma and kidney renin and prorenin were decreased in LL rats. In acute conditions, LL rats exhibited an unspecific hypersensitivity to AII and NE. Chronically given AII exerted a greater pressor effect in LL than in LN rats after 4 weeks (113 ± 3 vs 97 ± 5 mm Hg in LL and LN rats respectively, P < .05) and, even more, after 8 weeks of infusion (144 ± 9 vs 124 ± 4 mm Hg in LL and LN rats respectively, P < .05). The NE was devoid of chronic pressor effects. In conclusion, 1) the increased slow pressor response to AII may not be a critical pathogenetic factor in the development of hypertension, as it also exists in normotensive LL rats; 2) LN and LL rats have the same normal BP despite marked differences in their RAS, thus suggesting that there could be several forms of normotension as known for hypertension; and 3) the simple comparison between one genetically hypertensive strain and one single normotensive control strain does not allow one to conclude that a phenotypic difference is of pathophysiological significance. Am J Hypertens 2000;13:283–289 © 2000 American Journal of Hypertension, Ltd.

KEY WORDS: Lyon normotensive rats, Lyon low blood pressure rats, angiotensin II.

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Genetically hypertensive rats of the Lyon strains (LH) differ from their normoten-
sive (LN) controls by a low plasma and
kidney renin content contrasting with an
equisite sensitivity to the blood pressure (BP) lowering
effects of renin-angiotensin system (RAS) blockers
such as angiotensin converting enzyme (ACE) inhibi-
tors. Recently, we demonstrated that this paradoxical
association of a “low renin syndrome” and a high
response to ACE inhibitors could be explained by an
increased sensitivity of LH rats to the long-term pres-
sor effects of angiotensin II (AII).

Because, due to the drift that accompanies any ge-
etic selection, many of the phenotypic differences
that exist between one genetically hypertensive and
one genetically normotensive strain may well be un-
related to hypertension, we thought it of interest to
compare the biochemical and functional aspects of the
RAS in two genetically normotensive strains.

In that purpose, we took advantage of the fact that,
simultaneously to the selection of LH rats, two control
strains were obtained, the LN and the low blood pres-
sure (LL) rats. As LN and LL rats exhibit actually
similar mean BP levels, it was possible to conduct this
work in two generally pure and different normoten-
sive strains of rats that are close to LH rats. In that
respect, it is worth noting that the renin gene differs
between LN and LL rats, but not between LL and LH
rats.

METHODS

Animals Male LN and LL rats were housed in con-
trolled conditions (temperature: 21°C ± 1°C; humid-
ity: 60% ± 10%; lighting: 8 to 20 h) and received a
standard rat chow containing 0.3% sodium (AO3,
Usine d’Alimentation Rationnelle, Villemoisson-sur-
Orge, France). Part of the animals received the ACE
inhibitor perindopril (Servier Laboratories, Neuilly-
sur-Seine, France) at the dose of 3 mg/kg/day in
drinking water, from 3 weeks of age until the end of
the experiment. Perindopril concentration was ad-
justed weekly according to body weight and water
intake. All of the protocols were conducted in accor-
dance with our institutional guidelines concerning an-
imal care. Some of the data obtained in LN rats be-
longed to a previously published study.

Study 1: Biochemical Characteristics of the RAS

Plasma and kidney renin and prorenin were measured
in 11-week-old untreated male LN and LL rats using a
previously described method. Rats were killed by
decapitation 20 min after being tranquilized (diazep-
am 5 mg/kg, intraperitoneally) and blood was col-
lected on heparin at 4°C. Kidneys were dissected out,
weighted and stored at −80°C until assay. Plasma
prorenin concentration was defined as the difference
between total and active plasma renin concentrations.
For the total plasma renin assay, 40 μL of plasma were
incubated at 4°C in the presence of 20 μL of trypsin
treated with l-tosylamide phenylethyl chloromethyl
ketone (12 mg/mL, Worthington Biochemical, Free-
hold, NJ) for 1 h. At the end of this incubation period,
20 μL of soybean trypsin inhibitor (24 mg/mL, Sigma
Chemical, St. Louis, MO) were added and 25 μL of this
mixture incubated for 1 h at 37°C with 175 μL of
binaephrectomized rat plasma and 25 μL of tris(hy-
droxymethyl)-aminomethane HCL buffer containing
EDTA and phenylmethylsulfonyl fluoride. Each incu-
bation was performed in duplicate. Angiotensin I pro-
duction was measured by radioimmunoassay. For
the active renin assay, the protocol was the same but
the incubation with trypsin was omitted. For kidney
renin and prorenin content, a cortex sample (100 mg)
was thawed three times by changing temperature
from −20°C to 4°C and then homogenized with a
Polytron device (Kinematica, Lucerne, Switzerland).
After centrifugation (13,000 revolutions/min for 1 h),
the supernatant was collected and used for renin and
prorenin measurements as described above.

Study 2: Effects of Chronic ACE Inhibition

Groups of untreated and perindopril-treated LN and LL rats
were used. At 15 weeks of age, animals were instru-
mented for BP recording. Briefly, two polyethylene
catheters (PE50 fused to PE10) were inserted under
anesthesia (halothane 2% in oxygen), one via the left
femoral artery into the abdominal aorta and the other
via the left femoral vein into the inferior vena cava for
BP recording and intravenous injections, respectively.
The catheters were guided subcutaneously and exter-
rized at the back of the neck. After 24 h of post-
surgical recovery, the arterial catheter was connected to a
pressure transducer (Statham P23ID, Gould Inc,
Cleveland, OH) via a rotating swivel that allowed the
animals to move freely. Recording began 30 min after
connection to the transducer. The BP was digitized and
processed on-line by a computer (MVME SYS121,
Motorola Inc, Tempe, AZ) to determine and store
beat-to-beat values of systolic (SBP), diastolic (DBP),
and mean BP (MBP) as well as heart rate (HR). Off-line
data processing was performed by a workstation
(Sun Microsystems, Mountain View, CA).

Aortic BP and HR were recorded during a 1-h base-
line period. Then, untreated and perindopril-treated
animals received the ganglion-blocker chlorisondam-
ine (2.5 mg/kg intravenously, Novartis, Basel, Swit-
zerland) so as to suppress the baroreflex. Fifteen min-
utes after this administration, the venous catheter was
filled with AII (Sigma Chemical), and nine graded
doses of AII (1.25 to 320 ng/kg) were injected, sepa-
rated by time sufficient to allow BP and HR to return
to baseline values. The same procedure was used for
norepinephrine (NE) (Sigma Chemical), nine doses (from 10 to 2560 ng/kg) of which were administered. Changes in MBP were fitted using least-squares analysis to a sigmoidal logistic equation that provided estimates of the maximum effect and of ED$_{50}$. Data were retained for analysis when: 1) the $F$ value of the analysis of variance for regression gave a $P$ value inferior to 0.001; and 2) the calculated plateau did not differ by >30% from the MBP increase induced by the highest dose infused. Finally, both untreated and perindopril-treated rats having been ganglion-blocked by chlorisondamine received an intravenous bolus of a cocktail containing a V$_1$-vasopressin receptor antagonist, [β-mercapto-β, β-cyclopenta-methylene-propionyl,$^1$ O-Me-Tyr$^2$Arg$^8$]-vasopressin (10 μg/kg, Sigma Chemical), an α-adrenoceptor antagonist, phentolamine (5 mg/kg, Novartis), a β-adrenoceptor antagonist, propranolol (5 mg/kg, Novartis), perindopril (3 mg/kg, Servier Laboratories) and a nonspecific vasodilator, hydralazine (3 mg/kg, Sigma Chemical) to block the major pressor systems and to induce maximal vasodilatation. The BP level obtained 1 h after this procedure has already been shown to be a valuable index of intrinsic vascular resistances.$^{12,13}$ Finally, rats were killed with an overdose of pentobarbital sodium, and the left ventricle dissected out and weighed.

Study 3: Chronic Infusions of AII and NE in Perindopril-Treated Rats  

Four-Week AII Infusion  
Studies were conducted in LN and LL rats orally treated with perindopril from 3 weeks of age to the end of the experiment (see above) so as to eliminate the influence of variations in the endogenous production of AII. At 10 weeks of age, animals were housed in individual metabolic cages for a 1-week habituation period. They next received a sodium-free rat chow (Usine d’Alimentation Rationnelle, Na$_2$12, sodium content <4 mmol/kg) and a solution of sodium chloride (51 mmol/L in distilled water). At 11 weeks of age, sodium intake and excretion were measured for five consecutive days and the sodium balance calculated. Sodium concentration was measured by flame photometry (IL meter, model 243, Instrumentation Laboratory, Lexington, MA). At the age of 12 weeks, osmotic minipumps (Alzet 2ML4, Alza Corporation, Palo Alto, CA) were implanted subcutaneously under anesthesia (halothane 2% in oxygen) and connected to a polyethylene catheter (PE60 fused to PE10) inserted into the jugular vein. They delivered AII (Hypertensin, Novartis) at a constant rate of 200 ng/kg/min intravenously for 4 weeks. During the first 2 weeks of infusion, sodium balance was measured. During the last week of infusion (i.e., in 15-week-old rats), aortic BP was recorded during a 2-h baseline period in conscious, freely moving rats. After death by an overdose of pentobarbital sodium, the left ventricle was dissected out and weighed.

Eight-Week AII Infusion  
In additional groups of six perindopril-treated rats of the two strains, osmotic minipumps were implanted at 12 weeks of age. After 4 weeks of infusion, they were removed and replaced by new ones, to ensure another 4-week AII infusion period. At the end of week 8 of AII infusion, aortic BP was recorded beat-to-beat in freely moving animals during a 2-h period. Finally, rats were killed by an overdose of pentobarbital sodium, and the left ventricle was dissected out and weighed.

Four-Week NE Infusion  
Six rats of the two strains were treated with perindopril as described above. At the age of 12 weeks, NE dissolved in saline was infused during 4 weeks via minipumps at a constant rate of 1000 ng/kg/min. This dose of NE was chosen as it was, in acute conditions, equipressor to that of 200 ng/kg of AII (see results). At 15 weeks of age, aortic BP was recorded beat-to-beat during a 4-h period.

Statistical Analysis  
Values are means ± SEM. Comparisons between groups used one-way analysis of variance followed by a Fisher test. $P < .05$ was considered to be statistically significant.

RESULTS  

Biochemical Characteristics of the RAS  
Plasma renin and prorenin concentrations were lower in LL than in LN rats, this difference being significant only for prorenin (Table 1). A similar difference was found for kidney renin and prorenin contents reaching significance only for renin concentration. The ratio of the active renin to total renin did not differ between the two strains.

Effects of Chronic ACE Inhibition  
The LL rats had a slightly but significantly higher SBP than LN rats, whereas their DBP and MBP were not different (Table 2). The lower body weight (BW) of LN rats accounted for the increased relative left ventricle weight (LVW/BW) exhibited by that strain. The residual MBP used as an index of intrinsic vascular resistances did not differ between the two strains. Chronically given perindopril decreased MBP in a similar manner in the two strains (−23 and −24 mm Hg in LN and LL rats, respectively). It tended to decrease less markedly the relative left ventricle weight and the residual MBP in LN than in LL rats.

Acute Pressor Effects of AII and NE  
Figure 1 shows the dose-response curves for AII and NE. In untreated rats, the maximal effects of AII and NE were enhanced in LL rats compared to LN, this difference being significant only for AII. This was not associated with any
significant change in the ED_{50} for AII and NE. After perindopril treatment, the maximal pressor effect of AII and NE slightly decreased and no longer differed between the two strains. This contrasted with lower ED_{50} values in LL than in LN rats, which represented a nonspecific phenomenon as it affected the response to AII and NE.

Chronic Infusions of AII and NE in Perindopril-Treated Rats A 4-week infusion of AII increased significantly more BP in LL than in LN rats (Table 3). This difference was even greater after an 8-week infusion. The sodium balance (Figure 2), which did not significantly differ between LN and LL rats before the AII infusion, became positive in both strains for the first 24 h after its start. This sodium retention was followed the next day by a compensatory sodium loss that was more marked in LN than in LL rats. Then the sodium balance stabilized at the same level in the two strains during the following days. The 5-day cumulative sodium balances were not significantly different between the two strains during the AII infusion (891 ± 212 v 1103 ± 72 μmol for week 1, and 983 ± 146 v 1163 ± 121 μmol for week 2 of AII infusion in LN and LL rats respectively, NS). A 4-week NE infusion was devoid of pressor effect in both strains (95 ± 4 and 90 ± 5 mm Hg for MBP at the end of the infusion in LN and LL rats, respectively).

**DISCUSSION**

The present work demonstrates that two genetically close but different strains of normotensive rats can significantly differ in terms of biochemical and functional aspects of the RAS. This suggests that, as already accepted for hypertension, there exist several forms of normotension.

It is common to observe in hypertensive patients as well as in genetically hypertensive rats, such as adult SHR, and LH rats, the paradoxical association of low biochemical indices of RAS activity with an excellent antihypertensive effect of RAS blockers. Such a “low renin syndrome” remains to be explained.

In the present study, we wanted to determine whether this “low renin syndrome” existed uniquely in hypertensive, or whether it could also be observed in normotensive, rat strains. With that purpose, we compared the two genetically pure and different normotensive control strains (LN and LL rats), which have been simultaneously selected with LH rats.

**TABLE 1. BIOCHEMICAL CHARACTERISTICS OF THE RENIN-ANGIOTENSIN SYSTEM IN LYON RATS**

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Renal Cortex</th>
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<tbody>
<tr>
<td></td>
<td>Renin (ng AI/h/mL)</td>
<td>Prorenin (ng AI/h/mL)</td>
</tr>
<tr>
<td><strong>LN</strong></td>
<td>31.8 ± 3.6</td>
<td>53.7 ± 5.5</td>
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<tr>
<td><strong>LL</strong></td>
<td>23.7 ± 4.3</td>
<td>38.3 ± 4.1*</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

LN and LL, Lyon normotensive and low blood pressure rats, respectively; AI, angiotensin I; Pt, protein; Ratio, active renin to total renin (renin + prorenin).

* P < 0.05 LL versus LN rats.

**TABLE 2. CARDIOVASCULAR CHARACTERISTICS IN UNTREATED AND PERINDOPRIL-TREATED LYON RATS**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>MBP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>BW (g)</th>
<th>LVW/BW (mg/100 g)</th>
<th>Residual MBP (mm Hg)</th>
</tr>
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<tbody>
<tr>
<td><strong>Untreated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN</td>
<td>11</td>
<td>133 ± 2</td>
<td>97 ± 2</td>
<td>114 ± 2</td>
<td>341 ± 7</td>
<td>284 ± 4</td>
<td>213 ± 3</td>
<td>44 ± 1</td>
</tr>
<tr>
<td>LL</td>
<td>11</td>
<td>139 ± 2*</td>
<td>98 ± 2</td>
<td>117 ± 2</td>
<td>362 ± 5</td>
<td>330 ± 5*</td>
<td>201 ± 2*</td>
<td>41 ± 1</td>
</tr>
<tr>
<td><strong>Perindopril-treated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN</td>
<td>12</td>
<td>105 ± 3†</td>
<td>79 ± 3†</td>
<td>91 ± 3†</td>
<td>356 ± 7</td>
<td>257 ± 3†</td>
<td>185 ± 4†</td>
<td>39 ± 1†</td>
</tr>
<tr>
<td>LL</td>
<td>11</td>
<td>110 ± 2†</td>
<td>81 ± 1†</td>
<td>93 ± 1†</td>
<td>356 ± 5</td>
<td>297 ± 6†</td>
<td>164 ± 2†</td>
<td>35 ± 1†</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

LN and LL, Lyon normotensive and low blood pressure rats, respectively; SBP, DBP, and MBP, systolic, diastolic, and mean blood pressure, respectively; HR, heart rate; BW, body weight; LVW, left ventricular weight; Residual MBP (see text).

* P < 0.05 LL versus LN rats.

† P < 0.05 versus their own controls.
The renal synthesis and secretion of renin was measured using validated methods applied to plasma and kidney cortex extracts. As a whole, it appeared that the active to total renin ratio, used as an index of renin activation, did not differ between the two strains, whereas the synthesis and the release of renin were lower in LL than in LN rats. Similar findings were observed when LH rats were compared to LN controls either in vitro or in vivo, as well as in baseline conditions as after stimulation by BP decreases, isoprenaline infusions, or blockade of angiotensin AT \(_1\) receptors. Contrasting with this lower renin secretion rate in LL than in LN rats, an early and chronic ACE inhibition with perindopril exerted, in the two strains, similar effects on BP, relative left ventricle weight, and residual MBP used as an index of the overall structural vascular resistance. Once again, similar observations were made when LH rats were compared to LN controls. Because a likely explanation of the association of “low renin syndrome” and maintained antihypertensive efficacy of ACE inhibition is an increased sensitivity to the pressor effects of AII, this latter was studied in both acute and chronic conditions. Acutely, in untreated rats, we observed that ED\(_{50}\) for AII and NE did not differ between the two strains, whereas the maximal effect of AII was greater in LL than in LN rats. In perindopril-treated animals, LL rats exhibited a lower ED\(_{50}\) for both AII and NE. Thus, in acute conditions, no specific hypersensitivity to AII could be disclosed in LL rats. Chronic effects were studied in perindopril-treated animals so as to avoid any inter-

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**FIGURE 1.** Dose-response curves for angiotensin II and norepinephrine in untreated and perindopril-treated Lyon normotensive (LN) and low blood pressure (LL) rats. \(\Delta\)MBP, change in mean blood pressure; Max., maximal response; ED\(_{50}\), 50% effective dose. Numbers between brackets indicate the number of animals. Values are expressed as means \(\pm\) SEM. *P < 0.05 LL versus LN rats.
ference of spontaneous changes in the endogenous production of AII and to eliminate the possibility that baseline AII release may have been lower in LL than in LN rats. We report here that a 4-week and, even more, an 8-week infusion of AII increased significantly more BP in LL than in LN rats. As a consequence, LL rats exhibited an increased slow pressor response to AII when compared to LN animals. Similar findings were observed when LH rats were compared to LN controls. However, the differences were more marked than those reported here, as the MBP of LH rats established at 120 ± 6 and 176 ± 3 mm Hg after 4- and 8-week infusion of AII, respectively, whereas that of LL animals was, as reported above, 113 ± 3 and 144 ± 9 mm Hg. Sodium retention may not play a major role in the slow pressor response to AII, as cumulative sodium balances were similar in LN and LL strains. The lack of pressor response to a 4-week infusion of NE was likely explained by a desensitization of adrenoceptors as an acute bolus of NE normally increased BP in uninfused rats, but was devoid of effects in NE infused animals (data not shown).

Therefore, in qualitative terms, LL rats differ from LN controls in a manner to that of LH rats: both LL and LH rats respond to ACE inhibition despite a “low renin syndrome” and exhibit an increased response to chronic infusion of AII. Interestingly, it has to be noted that LL and LH rats bear the same renin gene allele, which differs from that of LN rats. Because the polymorphism found in the renin gene is situated within the first intron, our data suggest that this intron may involve regulatory elements, as already demonstrated for SHR.

In conclusion, the present work shows that different strains of rats can remain normotensive despite marked differences in their RAS, suggesting that there exist several forms of normotension. It also indicates that the comparison of one genetically hypertensive strain to a single normotensive strain is insufficient to safely approach the pathophysiology of high BP.

ACKNOWLEDGMENTS

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