Pivotal role of the renin-angiotensin system in Lyon hypertensive rats

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Lantelme, Pierre, Ming Lo, Laurent Luttenauer, and Jean Sassard. Pivotal role of the renin-angiotensin system in Lyon hypertensive rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1793–R1799, 1997.—We assessed the role of the renin-angiotensin system (RAS) in Lyon genetically hypertensive (LH) and normotensive (LN) rats by measuring 1) kidney renin and prorenin contents; 2) effects of early, prolonged angiotensin-converting enzyme (ACE) inhibition on blood pressure (BP) and regional hemodynamics; and 3) acute and chronic responses to angiotensin II (ANG II) and norepinephrine (NE). At the adult age, LH rats differed from LN rats by elevated BP, left ventricle weight, and vascular resistances, especially in the kidneys, associated with lower kidney renin and prorenin contents. ACE inhibition (perindopril, 3 mg·kg⁻¹·24 h⁻¹ orally from 3 to 15 wk of age) suppressed the development of hypertension, cardiac hypertrophy, and the increase in renal vascular resistances. No specific hypersensitivity to ANG II could be disclosed in acute conditions. In perindopril-treated LH rats, a 4-wk infusion of ANG II (200 ng·kg⁻¹·min⁻¹) but not of NE (1,000 ng·kg⁻¹·min⁻¹) restored hypertension, mimicked the hemodynamic alterations seen in untreated LH rats, and produced a brief sodium retention. It is concluded that in LH rats, despite a low basal renin secretion, hypertension and hemodynamic abnormalities 1) are fully dependent on an active RAS and 2) may involve an enhanced sensitivity to the chronic effects of ANG II.

hemodynamics; angiotensin-converting enzyme inhibition; angiotensin II

THE RENIN-ANGIOTENSIN SYSTEM (RAS) is a major determinant of blood pressure (BP) through its action on vascular resistances and renal sodium handling. The efficacy of angiotensin-converting enzyme (ACE) inhibition (4, 9), antirenin immunization (16), or antagonism of angiotensin II (ANG II) subtype 1 (AT₁) receptors (21) suggests that an active RAS is required for the development of genetic hypertension in spontaneously hypertensive rats (SHR). However, biochemical indexes of RAS activity, such as renal renin release, plasma renin, and ANG II concentrations, are normal or decreased in adult SHR (2, 10, 18, 25, 26). To explain this paradox, it has been suggested that SHR exhibit an increased sensitivity to the pressor effects of ANG II (11).

We investigated whether this pattern (low or normal biochemical indexes of RAS activity, efficacy of ACE inhibition, and increased sensitivity to ANG II) applied to Lyon genetically hypertensive (LH) rats. Adult LH rats exhibit a low plasma renin concentration (1, 30), a decreased renal renin release (19), and a greater renal responsiveness to acute infusion of ANG II (14) compared with their simultaneously selected, normotensive counterparts, the Lyon normotensive (LN) rats. These functional characteristics of the RAS are associated with differences in the renin gene (22, 24).

To evaluate the functional importance of the RAS in the Lyon model, we measured 1) the kidney renin and prorenin contents, 2) the BP and regional hemodynamics in control conditions or after an early and prolonged blockade of the RAS, and 3) the acute and chronic pressor effects of ANG II in RAS-blocked animals.

MATERIALS AND METHODS

Animals

Male LH and LN rats belonging to the 51st generation of the strains were housed in controlled conditions (temperature: 21 ± 1°C; humidity: 60 ± 10%; lighting: 0800–2000) and received a standard rat chow (NA A03; Usine d'Alimentation Rationnelle, Villemonais-sur-Org, France). The ACE inhibitor perindopril (Servier Laboratories, Neuilly-sur-Seine, France) was given at the dose of 3 mg·kg⁻¹·day⁻¹ (8) in drinking water, from 3 wk of age up to the end of the experiments. Its concentration was adjusted weekly according to body weight and water intake.

All the protocols were conducted in accordance with our institutional guidelines concerning animal care.

Methods

Renin and prorenin assays. For kidney renin and prorenin measurements, a cortex sample (100 mg) was thawed three times by changing the temperature from −20 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. Prorenin concentration was defined as the difference between activated renin (total renin) and active renin concentrations. For the activated kidney renin assay, 40 µl of supernatant were incubated at 4°C in the presence of 20 µl of trypsin treated with L-1-tosylamide 2-phenylethyl chloromethyl ketone (12 g/ml; Sigma Chemical, St. Louis, MO) were added and 25 µl of this mixture were incubated for 1 h at 37°C with 175 µl of rabbit plasma and 25 µl of tris(hydroxymethyl)-aminomethane·HCl buffer containing EDTA and phenylmethylsulfonyl fluoride. Each incubation was performed in duplicate. Angiotensin I (ANG I) production was measured by radioimmunoassay (20). For the active kidney renin assay, the protocol was the same but the incubation with trypsin was omitted.

BP recording in freely moving rats. BP and heart rate (HR) were measured in freely moving animals with the use of our computerized technique (6). Briefly, two polyethylene catheters (PE-50 fused to PE-10) were inserted under anesthesia (2% halothane in oxygen), one via the left femoral artery in...
the abdominal aorta and one via the left femoral vein in the inferior vena cava for BP recording and intravenous injections, respectively. The catheters were guided subcutaneously and exteriorized at the back of the neck. After 24 h of postsurgical recovery, the arterial catheter was connected to a pressure transducer (Statham P231D; Gould, Cleveland, OH) via a rotating swivel that allowed the animals to move freely. Recording began 30 min after connection to the transducer. BP was digitized and processed on-line by a computer (MVME SYS121; Motorola, Tempe, AZ) to determine and store beat-to-beat values of systolic (SBP), diastolic, and mean BP (MBP), as well as HR. Off-line data processing was performed by a work station (Sun Microsystems, Mountain View, CA).

Cardiac output and regional blood flow measurement. Systemic and regional hemodynamics were assessed in freely moving rats using radiolabeled microspheres (23). With the rats under halothane anesthesia, a catheter (PE-10) was inserted into the left ventricle via the right carotid artery. Correct placement within the ventricle was confirmed by the appearance of a characteristic left ventricular pressure waveform. After 5 h of recovery, the aortic catheter was connected to a pressure transducer to measure the BP level before the injection of microspheres. It was then disconnected, and a reference blood sample was withdrawn at a constant rate of 0.6 ml/min with a motor-driven syringe for 2 min. Ten seconds after arterial blood sampling had begun, cobalt-57-labeled microspheres (15.5 ± 0.1 µm; DuPont-NEN Products, Boston, MA) suspended in 0.5 ml of a 63% sucrose solution were injected into the left ventricle over a period of 25 s. Rats were then killed with an overdose of pentobarbital sodium. Kidneys, spleen, small intestine, heart, brain, and hindlimb muscle were removed, weighed, and placed into a 10% Formol solution, and their radioactivity was counted (Cobra 5010; Packard, Downers Grove, IL). The correct position of the left ventricular catheter was rechecked at the time of cardiac dissection. Cardiac output was calculated as (total counts injected/counts in the reference sample) × reference sample flow rate; cardiac index was calculated as cardiac output/body weight; and total vascular resistance was calculated as mean arterial pressure/cardiac index. Individual organ blood flows were calculated as (counts per gram of tissue/counts in the reference sample) × reference sample flow rate, and organ arterial vascular resistance was calculated as mean arterial pressure/organ flow rate.

Sodium balance Animals were housed in individual metabolic cages and received a rat chow (NA 212, Usine d’Alimentation Rationnelle) containing less than 4 mmol/kg (d’Alimentation Rationnelle) indicating that BP increases remained within the physiological range. Sodium balances were measured during the 2 wk of age to the end of the experiment (see above). At 11 wk of age, sodium balances were assessed, and indirect SBP was measured weekly. At 12 wk, osmotic minipumps (ALZET 2ML4; ALZA, Palo Alto, CA) were implanted subcutaneously under halothane anesthesia (2% in oxygen) and connected to a polyethylene catheter (PE-60 fused to PE-10) inserted into the jugular vein. They delivered ANG II (Hypertensin, Ciba-Geigy) at a constant rate of 200 ng·kg⁻¹·min⁻¹·iv for 4 wk. This dose was selected from the literature (27) and from pilot experiments indicating that BP increases remained within the physiological range. Sodium balances were measured during the 2 following weeks. During the last week of infusion (i.e., in 15-wk-old rats), aortic BP was recorded during a 2-h baseline period. After euthanasia with pentobarbital sodium, the left ventricle was dissected out and weighted.

In two additional groups of similarly treated LH and LN rats, cardiac output and regional hemodynamics were assessed using the microspheres technique, at the end of the 4-wk ANG II infusion.

To assess the specificity of the observed effects, a similar protocol was followed using an infusion of NE. Perindopril-treated animals of both strains were infused with NE (Sigma) at a constant rate of 1,000 ng·kg⁻¹·min⁻¹, a dose that, when
administered acutely, produced a similar BP elevation to a bolus injection of 200 ng/kg of ANG II (see RESULTS). At 15 wk of age, aortic BP was recorded beat-to-beat during a 4-h baseline period. Next, a bolus of NE (1,000 ng/kg) was injected so as to assess the acute response to this agent. Finally, at the end of the infusion, the remaining content of the minipumps was sampled and tested. It was found to be as pressor as a freshly prepared NE solution, thus ruling out any destruction of NE during the infusion period.

Statistical Analysis

Values are expressed as means ± SE. Comparisons between groups used one-way analysis of variance followed by a Fisher test. \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Biochemical Characteristics of the RAS in Lyon rats

LH rats had lower kidney renin and prorenin contents than LN rats (Table 1). The active renin-to-total renin ratio, used as an index of renin activation, was similar in the two strains.

<table>
<thead>
<tr>
<th>Renal Cortex</th>
<th>Renin, µgANG I·h⁻¹·mg protein⁻¹</th>
<th>Prorenin, µgANG I·h⁻¹·mg protein⁻¹</th>
<th>Ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH 10</td>
<td>1.93 ± 0.14*</td>
<td>2.11 ± 0.19*</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>LN 10</td>
<td>3.20 ± 0.37</td>
<td>3.67 ± 0.23</td>
<td>0.46 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = \) no. of animals. LH and LN, Lyon hypertensive and Lyon normotensive strain, respectively; ANG I, angiotensin I; ratio, renin-to-total renin (renin + prorenin) ratio. \( *P < 0.05 \) vs. LN.

Table 1. Kidney renin and prorenin levels in 11-wk-old male rats of the Lyon strains

Hemodynamic Effects of Chronic ACE inhibition

Figure 1 shows that, in control animals, indirect SBP values were higher in LH than in LN rats starting from 6 wk of age. At 15 wk of age, hypertension had stabilized, which was confirmed by aortic BP recordings (Table 2). The hemodynamic profile of LH rats associated a normal cardiac output with markedly increased regional vascular resistances, especially in the renal (+99%), splenic (+92%), and mesenteric (+69%) vascular beds, which resulted in an increased total vascular resistance. Renal vasoconstriction was associated with a decreased blood flow, which was also the case in the splenic and cerebral circulations. Hypertension was associated with cardiovascular damages (Table 2), as assessed by a significant left ventricle hypertrophy and elevated residual MBP used as an index of intrinsic vascular resistances (50 ± 2 vs. 44 ± 1 mmHg in LH and LN rats, respectively, \( P < 0.05 \)).

Table 2. Cardiovascular characteristics in control, perindopril-treated, and perindopril-treated, ANG-II-infused rats of the Lyon strains

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>MBP, mmHg</th>
<th>HR, beats/min</th>
<th>LV Wt/Body Wt, mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control LH 11</td>
<td>175 ± 3*</td>
<td>122 ± 4*</td>
<td>145 ± 4*</td>
<td>357 ± 6</td>
<td>223 ± 4*</td>
<td></td>
</tr>
<tr>
<td>Control LN 11</td>
<td>133 ± 2</td>
<td>97 ± 2</td>
<td>114 ± 2</td>
<td>341 ± 7</td>
<td>213 ± 3</td>
<td></td>
</tr>
<tr>
<td>Perindopril LH 12</td>
<td>113 ± 3*</td>
<td>81 ± 2</td>
<td>94 ± 2</td>
<td>360 ± 7</td>
<td>168 ± 2*</td>
<td></td>
</tr>
<tr>
<td>Perindopril LN 12</td>
<td>105 ± 3</td>
<td>79 ± 3</td>
<td>91 ± 3</td>
<td>356 ± 7</td>
<td>185 ± 4</td>
<td></td>
</tr>
<tr>
<td>Perindopril + ANG II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH 10</td>
<td>152 ± 8*</td>
<td>99 ± 6*</td>
<td>120 ± 6*</td>
<td>354 ± 6*</td>
<td>217 ± 7*</td>
<td></td>
</tr>
<tr>
<td>LN 11</td>
<td>117 ± 6</td>
<td>83 ± 4</td>
<td>97 ± 5</td>
<td>381 ± 6</td>
<td>190 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. SBP, DBP, and MBP, systolic, diastolic, and mean blood pressure, respectively; HR, heart rate; LV, left ventricle. \( *P < 0.05 \) vs. LN.

Fig. 1. Indirect systolic blood pressure (SBP), cardiac output (CO), total vascular resistances (TVR), regional blood flows, and vascular resistances in control Lyon hypertensive (LH) and normotensive (LN) rats. n, No. of animals. \( *P < 0.05 \) vs. LN rats.
Chronic perindopril treatment (Fig. 2) totally suppressed the interstrain difference in indirect SBP, and only a slight difference still subsisted for aortic SBP (Table 2). Cardiac output became higher in LH than in LN rats, and there were no longer differences in the regional blood flows and vascular resistances. BP normalization was associated with a decrease in residual MBP, which became significantly lower in LH (33 ± 1 mmHg) than in LN (39 ± 1 mmHg) rats. The efficiency of ACE inhibition was demonstrated by the nearly complete suppression of pressor responses to ANG I (changes in MBP were 6 ± 2 and 5 ± 2 vs. 41 ± 3 and 33 ± 3 mmHg in perindopril-treated and control LH and LN rats, respectively).

Acute Pressor Effects of ANG II and NE

In conscious, ganglion-blocked animals, the maximum response to ANG II was greater in LH than in LN rats, and ED50 was very close (Fig. 3). Similar results were found for NE. In perindopril-treated animals, the differences in the maximum pressor responses to ANG
II and NE seen in controls disappeared. Perindopril increased the ED50 for ANG II and NE in LN but not in LH rats.

Responses to Chronic ANG II and NE Infusions in Perindopril-Treated Rats

A 4-wk ANG II infusion raised BP and left ventricle weight significantly more in perindopril-treated LH than in LN rats (Table 2). In addition, Fig. 4 shows that this infusion restored the hemodynamic pattern seen in untreated LH and LN rats of the same age, i.e. similar cardiac output and increased total vascular resistances. In these ANG II-infused as well as in untreated LH rats, the most marked increases in vascular resistances were observed in the splanchnic area (kidneys: +59%; spleen: +64%, mesentery: +52%), whereas no difference in regional blood flows could be discerned. The sodium balance (Fig. 5), which did not differ among LH and LN rats before the ANG II infusion, became positive in both strains for 24 h after its start. Interestingly, this sodium retention was followed the next day by a compensatory sodium loss in LN but not in LH rats, the sodium balances stabilizing at the same level in the two strains during the following days.

In identical conditions, a 4-wk infusion of NE did not raise BP in perindopril-treated LH and LN rats (97 ± 7 and 114 ± 3 mmHg for SBP in 6 LH and 6 LN rats, respectively). At the end of the infusion period, rats given an intravenous injection of 1,000 ng/kg of NE exhibited much lower pressor effects (35 ± 3 and 32 ± 1 mmHg in LH and LN rats, respectively) than non-NE-infused animals (see Fig. 3).

DISCUSSION

The present work demonstrates that the high BP and the associated splanchnic vasoconstriction exhibited by LH rats disappear after early chronic ACE inhibition and are mimicked by a prolonged infusion of ANG II. This suggests that despite the low renin secretion seen in adult LH rats, high BP in this strain depends on an active RAS and, more specifically, may be related to an increased sensitivity to the long-term pressor effects of ANG II.

The efficacy of pharmacological blockade of the RAS has been repeatedly observed in genetically hypertensive rats (4, 9, 16, 21), thus suggesting a critical role of the RAS in the pathogenesis of these forms of hypertension. However, this efficacy contrasts with the finding that in adult hypertensive animals such as the SHR, the biochemical indexes of renin secretion are usually normal or decreased (10, 25, 26), and renal renin release following a decrease in renal perfusion pressure is low (28). Such a discrepancy between the efficacy of RAS blockade and a low plasma renin activity has also been observed in some patients with essential hypertension (31). This led Li and Jackson (11) to hypothesize
that genetic hypertension may involve a greater sensitivity to the pressor and/or the trophic effects of ANG II. Because of their simultaneous selection from a single restricted colony of Sprague-Dawley rats, LH animals and their LN controls are closely related in terms of genetics, as demonstrated by an allelic difference, which was established as 11% between the two strains (3). In these conditions and because they harbor a different renin gene allele (22, 24), the Lyon rats seem of special interest for studying the role of the RAS in the development of spontaneous hypertension. In the present experiment, we observed that adult LH rats exhibited significantly lower renal renin and prorenin levels than LN ones. This is in accordance with previous reports showing a similar interstrain difference in plasma renin activity (L, 30). Because the renal renin-to-total renin ratio did not differ among LH and LN rats, it can be suggested that, in LH rats, the low renin levels cannot be accounted for by an abnormal prorenin processing. Associated with this low renin activity, LH rats differed from LN by elevated BP, left ventricle relative weight, and residual mean BP obtained after blockade of the major constrictor pathways, an index of intrinsic resistances (15, 29). These two latter parameters indicate that cardiac and vascular remodeling developed in LH rats. As usual in genetic hypertension (5), the cardiac output was similar in the two strains, whereas in LH rats there was a twofold increase in total vascular resistances. This was especially marked in the kidneys, where a decreased flow was observed. This finding is in accordance with previous observations made in vitro (13) and in anesthetized rats (12) showing the existence of a preglomerular vasoconstriction in LH kidneys, which likely contributes to the shift of pressure-natriuresis toward high BP, a prerequisite for a stable hypertension (7) already observed in LH rats (12).

An early chronic ACE inhibition with perindopril prevented the rise of BP in LH rats and the associated cardiac hypertrophy and elevated residual mean BP. The BP normalization was associated with a fall in total vascular resistances and the disappearance of the splanchnic vasoconstriction. Consequently, the development of hypertension in LH rats requires an active RAS. This suggests that LH animals could exhibit exaggerated responses to ANG II. Such an hypothesis of an increased sensitivity to ANG II was assessed in both acute and chronic conditions. NE responses were measured in the same conditions so as to evaluate the specificity of the observed changes. In acute conditions, the dose-response curves measured in conscious ganglion-blocked rats showed that the maximum effect of ANG II and of NE was higher in LH than in LN rats, whereas the corresponding ED50 did not significantly differ. This suggests an unspecific increased responsiveness to constrictors that may be related to vascular hypertrophy in LH rats because it disappeared in perindopril-treated animals.

We then studied the responses to 4-wk infusions of ANG II or of NE in perindopril-treated LH and LN rats. In these conditions, NE was unable to elevate the BP of either LH or LN rats. This lack of effect could not be explained by a degradation of NE in the minipumps, because the constrictor effect of the solution remaining in the minipumps after the infusion was identical to that of a freshly prepared NE solution. More probably, it was due to a downregulation of adrenoceptors, as demonstrated by the fact that a bolus of NE was much less pressor in NE-infused than in noninfused LH and LN rats. On the contrary, ANG II induced a progressive increase in BP that was larger in LH than in LN rats. Higher BP was then associated with an increase in vascular resistances, which was prominent in the splanchnic area and, above all, in the kidneys, thus mimicking the hemodynamic pattern observed in untreated LH rats. This result fits well with a previous study performed in anesthetized animals showing an increased renal responsiveness of LH kidneys to acute infusion of ANG II (14). Consistent with this renal vasocostriction, a transient but significant sodium retention was observed despite similar BP levels, suggesting a rightward shift of the pressure-natriuresis relationship.

In conclusion, with the use of chronic protocols followed by measurements in freely moving and, as far as possible, unstressed adult rats, it was observed that the paradox (low renin secretion and full antihypertensive efficacy of ACE inhibition) seen in LH rats cannot be explained by selective increase in the sensitivity to the acute effects of ANG II but more likely by an enhanced response to the long-term, slow pressor action of ANG II. This action may involve a general increase of vascular resistances due to cardiovascular remodeling, possibly favored by the trophic effects of ANG II. In addition, the most exaggerated response to chronic ANG II infusion was observed in the renal circulation and was probably crucial to maintaining a stable hypertension in LH animals, according to Guyton's theory (7). Interestingly, this study also demonstrates that in genetically hypertensive rats as well as in some hypertensive patients, RAS blockade may be efficient even in the presence of low values of the biochemical indexes of RAS activity.

Perspectives

Despite numerous attempts to explain it, the relationship between the RAS and hypertension remains unclear. The present study deals with a model of genetic hypertension that associates a low renin secretion rate with an exquisite sensitivity to RAS blockade. Such a "renin paradox," which can be observed in other animal models of hypertension as well as in hypertensive patients, suggests either an inappropriate release of renin or an enhanced sensitivity to the effects of ANG II. The present work favors the second hypothesis by demonstrating that in genetically hypertensive rats of the Lyon strain, the long-term pressor effects of ANG II are specifically enhanced. This long-term potentiation seems to be a unique property of ANG II. Whatever its mechanisms, it may well be crucial to explain the major influence of the RAS on the long-term blood pressure control.
REFERENCES


