Pressure Independence of Renin Release by Isolated Kidneys of Lyon Hypertensive Rats

Isac A. Medeiros, Madeleine Vincent, Daniel Benzoni, and Jean Sassard

In the present work the influence of perfusion pressure on renal functions and renin release was studied before and after the blockade of thromboxane A₂/prostaglandin H₂ (TXA₂/PGH₂) receptors using isolated kidneys from 7-week-old genetically hypertensive (LH), normotensive (LN), and low blood pressure (LL) rats of the Lyon strain. Kidneys were single pass perfused with Krebs-Henseleit solution with a gelatine derivative (Polygeline) added as an oncotic agent. A servocontrolled system stabilized the renal perfusion pressure (RPP) at any chosen (±1 mm Hg) level. In baseline conditions (RPP, 90 mm Hg), LH (n=7) kidneys differed from LN (n=6) and LL (n=8) controls by increased vascular resistance, decreased glomerular filtration rate, and natriuresis. The LH kidney responses to stepwise changes in RPP (between 60 and 170 mm Hg) differed from those of LN and LL rats by a significantly lower perfusion flow, glomerular filtration rate, and natriuresis. Above all, the reduction in RPP, which induced a marked and highly reproducible renin release in LN and LL kidneys, was devoid of effects in LH kidneys. The blockade of TXA₂/PGH₂ receptors by AH23848 (4x10⁻⁹ M) did not change the baseline (RPP, 90 mm Hg) functions of kidneys of the three strains. During changes in RPP, the responses of LN and LL kidneys were not modified, whereas LH kidneys exhibited significant increases in both glomerular filtration rate and natriuresis. Finally, AH23848 significantly decreased the renin release by kidneys of the three strains. It is concluded that renin release of LH isolated kidneys is independent of the perfusion pressure and that renal TXA₂/PGH₂ receptor activation participates in the renin release and in the altered functions exhibited by kidneys of LH rats. (Hypertension 1992;19:582-588)

KEY WORDS • kidney • renin • natriuresis • thromboxane A₂ • renal function • Lyon rat • genetic hypertension

Several recent experiments using kidney transplantation¹,² have emphasized the primary role played by the kidney in the pathogenesis of genetic hypertension. Obviously, among the pressor factors originating in the kidney, the most important is the renin-angiotensin system. In Lyon genetically hypertensive (LH) rats, the plasma renin activity is normal in young animals and low in adult ones.³ Similar findings have been reported in Japanese spontaneously hypertensive rats (SHR).⁴ Despite these low levels of plasma renin, both models are highly sensitive to the blockade of the renin-angiotensin system.⁵ This demonstrates that, in genetically hypertensive rats, the low renin levels remain inappropriate and actively contribute to blood pressure elevation.

Such an inappropriate renin secretion may reflect a polymorphism of the renin gene, as found in Dahl salt-sensitive rats,⁷ or an abnormal control by its physiological regulators. Because the renin gene polymorphism in LH rats does not appear directly related to the blood pressure level,⁸ we thought it of interest to assess the control of renin secretion by one of its major regulatory factors, the renal perfusion pressure (RPP). In addition, since in previous experiments we have shown that kidneys of LH rats synthesized an excess of thromboxane A₂ (TXA₂),⁹ a vasoconstrictor prostaglandin that may be involved in renin secretion,¹⁰ the baroreceptor control of renin secretion was studied before and after blockade of TXA₂/prostaglandin H₂ (PGH₂) receptors. All these experiments were conducted in isolated perfused kidneys from LH rats and its two control strains.¹¹

Methods

Animals

Seven-week-old male LH, normotensive (LN), and low blood pressure (LL) rats of the Lyon strain were used. Animals were housed under constant conditions of temperature (21±1°C), lighting (8 AM to 8 PM), and humidity (60±10%). They were fed a standard diet (Elevage UAR, Villemoisson s/ Orge, France) and had free access to tap water. Systolic blood pressure (SBP) was measured on 2 consecutive days before the experiments by a plethysmographic method (Narco BioSystems, Austin, Tex.) in the prewarmed (37°C for 10 minutes), unrestrained conscious rats.

Kidney Preparations

In sodium pentobarbital (45 mg/kg i.p.)-anesthetized rats, the right kidney was isolated according to Schmidt

From the Department of Physiology and Clinical Pharmacology, URA CNRS 606, Faculty of Pharmacy, Lyon, France.
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Address for correspondence: Pr. J. Sassard, Faculté de Pharmacie, 8, avenue Rockefeller, 69373, Lyon Cedex 08, France.
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FIGURE 1. Line plots show renin release (RR) from Lyon normotensive isolated kidneys: Influence of the duration and the pressure of perfusion (RPP). Al, angiotensin I. *p<0.05 vs. values obtained at RPP of 120 mm Hg.

Kidney Perfusion

The perfusion solution was a blood-free modified Krebs-Henseleit solution containing 35 g/1 of a gelatine derivative (Polygeline, Behring, Marburg, FRG) as an oncotic agent. The composition of the perfusion medium has been described elsewhere. Just before use, the perfusate was filtered through 2 millipore filters (1.2 μm and 0.8 μm, successively) and 0.5 g/l polyfructosan was added (Inutest, Laevosan, Linz, Austria). This solution, maintained at 37°C, was continuously bubbled with a 95% O₂-5% CO₂ mixture and single pass perfused using a peristaltic pump (Minipuls 2, Gilson, Paris). The RPP (mm Hg), monitored through a pressure transducer (model P231D, Statham Instrument Division, Gould Inc., Oxnard, Calif.), fed the pump through a specially designed device that stabilized the pressure at any chosen level (± 1 mm Hg) by servocontrolling the perfusion flow.

Renal Function Parameters

Renal perfusion flow (RPF) (ml/min/g) and urinary flow rate were measured by weighting. Renal vascular resistances (mm Hg/ml/min/g) were calculated as the ratio RPP/RPF. Glomerular filtration rate (GFR) (ml/min/g) was measured by polyfructosan clearance (Technicon) and sodium concentration by flame photometry (IL meter 243). Renin concentration in the venous effluent was measured by the radioimmunoassay of angiotensin I (Ang I) generated during incubation at pH 6.5 with binephrectomized rat plasma used as substrate according to Menard and Catt. For each venous sample, Ang I generation was measured after three incubation times to ensure the excess of substrate. Renin release (ng Ang I/hr/min) was calculated by multiplying renin concentration by the flow rate.

Protocols

Influence of renal perfusion pressure. Preliminary studies using LN kidneys demonstrated (Figure 1) that renin release from isolated kidneys perfused at 80 mm Hg remained stable for 2 hours and reductions in RPP from 120 to 50 mm Hg induced a rapid and reproducible renin release, which returned to baseline within 15 minutes after reestablishing RPP at 120 mm Hg. According to these data, the influence of RPP on the renin release was studied as follows: after a 30-minute stabilization period during which the kidneys were perfused at 90 mm Hg, venous effluent and urine samples were obtained to determine the baseline values. Then RPP was set at 170 mm Hg and was progressively decreased to 150, 130, 110, 85, 80, 75, and 60 mm Hg. Each level of RPP was maintained for 5 minutes. Venous samples were drawn after 4 minutes, and urine samples were collected during the last 4 minutes. After weighting, samples were kept at -20°C until assay.

Effects of a TXA₂/IPGH₂ Receptor Blockade on Renal Response to Changes in Perfusion Pressure. At the end of the previous protocol, the kidneys were perfused with AH23848 (a gift from Glaxo, Greenford, England), a TXA₂/IPGH₂ antagonist, at a final concentration of 4×10⁻⁶ M according to Liu et al. After a 15-minute stabilization period (RPP, 90 mm Hg), RPP was stepwise changed from 170 to 60 mm Hg according to the protocol described above. Venous effluent and urine...
samples were obtained and processed similarly. At the end of the study, the servocontrol of RPP was stopped, and the efficiency of the receptor blockade was demonstrated by the virtual disappearance (3.5±0.6, 4.8±0.8, and 4.0±0.6 mm Hg in LN, LL, and LH kidneys, respectively) of the pressor effects of U46619 (Sigma Chemical Co., St. Louis, Mo.), an agonist of TXA2/PGH2 receptors. U46619 was infused for 3 minutes at a concentration of 2.7×10⁻⁷ M, which was shown in preliminary experiments to induce submaximal increases in RPP (78±8 mmHg, n=4) in isolated LN kidneys untreated with AH23848.

Influence of \( \beta \)-adrenergic receptor stimulation on the renin release from kidneys of Lyon hypertensive rats. To verify that LH kidneys could release renin in response to a different stimulus than RPP, we studied, in addition to AH23848, the extent of the receptor blockade was demonstrated by the virtual disappearance (3.5±0.6, 4.8±0.8, and 4.0±0.6 mm Hg in LN, LL, and LH kidneys, respectively) of the pressor effects of U46619 (Sigma Chemical Co., St. Louis, Mo.), an agonist of TXA2/PGH2 receptors. U46619 was infused for 3 minutes at a concentration of 2.7×10⁻⁷ M, which was shown in preliminary experiments to induce submaximal increases in RPP (78±8 mmHg, n=4) in isolated LN kidneys untreated with AH23848.

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Results

Baseline Characteristics

Seven-week-old LH rats exhibited a significantly higher indirect SBP (Table 1) than the LN and LL controls, both of which, as usual, did not differ in that respect. Since the body and kidney weights were higher in LH rats than in LN and LL controls, all the parameters were corrected for kidney weight. It must be emphasized that the interstrain differences in kidney weight were too small to account for the marked changes in renal functions described below. After 30 minutes of equilibration, the isolated kidneys perfused at a pressure of 90 mm Hg exhibited marked interstrain differences. LH kidneys differed from LN by a significantly lower urinary sodium excretion (\( \text{UN,V} \)). LH kidneys, compared with LN and LL controls, exhibited increased vascular resistance and lower renin release and GFR. This latter accounted for most of their low natriuresis since the sodium reabsorption rate (% of filtered sodium) did not differ among strains.

Influence of Perfusion Pressure on Renal Function and Renin Release

As shown by Figure 2, any increase in RPP was associated with increases in the perfusate flow and decreases in sodium reabsorption rate, which were identical in LN and LL kidneys. GFR and natriuresis increased also but more markedly in LN than in LL kidneys. Since the sodium reabsorption rate did not differ between these two strains, the more marked pressure–natriuresis observed in LN rats is related to an elevated filtration of sodium. Although the trend of the pressure effects was similar in LH kidneys, these latter strikingly differed from both LN and LL controls by a much lower elevation in flow, GFR, and natriuresis. At any perfusion pressure level above 130 mm Hg the

TABLE 1. Baseline Values Obtained in 7-Week-Old Rats of the Lyon Strains Before and After Thromboxane A2/Prostaglandin H2 Receptor Blockade With AH23848

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LL (n=8)</th>
<th>LN (n=6)</th>
<th>LH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>119±3</td>
<td>119±3</td>
<td>150±5†</td>
</tr>
<tr>
<td>BW (g)</td>
<td>155±5</td>
<td>138±7</td>
<td>191±8†</td>
</tr>
<tr>
<td>LKW (g)</td>
<td>0.73±0.02</td>
<td>0.77±0.03</td>
<td>0.89±0.05†</td>
</tr>
<tr>
<td>LKW/BW (%)</td>
<td>0.47±0.02</td>
<td>0.56±0.03</td>
<td>0.47±0.03</td>
</tr>
<tr>
<td>RVR (mm Hg/ml/min/g)</td>
<td>8.2±0.3</td>
<td>8.3±0.4</td>
<td>14.9±0.9†</td>
</tr>
<tr>
<td>GFR (ml/min/g)</td>
<td>0.47±0.02</td>
<td>0.54±0.03</td>
<td>0.40±0.01†</td>
</tr>
<tr>
<td>( \text{UN,V} ) (µeq/min/g)</td>
<td>7.7±0.5*</td>
<td>12.7±2.1</td>
<td>5.9±0.5†</td>
</tr>
<tr>
<td>( \text{RN} ) (%)</td>
<td>89±1</td>
<td>85±2</td>
<td>89±1</td>
</tr>
<tr>
<td>RR (ng Ang I/hr/min)</td>
<td>534±44</td>
<td>561±37</td>
<td>176±19†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LL, Lyon low blood pressure rats; LN, Lyon normotensive rats; LH, Lyon hypertensive rats; SBP, systolic blood pressure; BW, body weight; LKW, left kidney weight; RVR, renal vascular resistance; GFR, glomerular filtration rate; \( \text{UN,V} \), urinary sodium excretion; \( \text{RN} \), sodium reabsorption rate; RR, renin release; Ang I, angiotensin I.  
*\( p<0.05 \) vs. LN.  
†\( p<0.05 \) vs. LL.
sodium reabsorption of LH kidneys was higher than that of LL kidneys.

The relation between RPP and renin release is shown in Figure 3. In LN and LL kidneys the maximum release was observed at RPP of 85 mm Hg and then progressively decreased when RPP was elevated up to 170 mm Hg. Surprisingly, there was no evidence of this well-known baroreceptor control of renin release in LH kidneys. The renin release of LH kidneys remained stable at values significantly lower than those of LN and LL kidneys up to 110 mm Hg and reached higher values at a RPP level of 170 mm Hg.

Effects of TXA$_2$/PGH$_2$ Receptor Blockade

Table 1 indicates that, in baseline conditions, a 15-minute infusion of AH23848 (4×10$^{-6}$ M) did not significantly alter the characteristics of the kidneys of the three strains. However in the three strains, vascular resistances, renin release, and sodium reabsorption rate tended to decrease while the urinary excretion of sodium slightly increased. As illustrated in Figure 4, the overall effects of AH23848 were to increase GFR and natriuresis and to decrease renin release in response to changes in RPP. In LN kidneys, only the renin release was significantly decreased by AH23848. In LL kidneys this decrease in renin release was more marked and was associated with an enhanced natriuresis at the perfusion pressure of 170 mm Hg. On the other hand, LH kidneys infused with AH23848 exhibited significant increases in GFR and natriuresis. The increase in natriuresis was more marked than that of GFR and therefore was partly accounted for by a decreased sodium reabsorption. Finally, AH23848 significantly lowered the renin release of LH kidneys, this latter remaining independent of the pressure level.

Influence of Isoproterenol on Renin Release From Kidneys of Lyon Hypertensive Rats

While the perfusion pressure was maintained at 90 mm Hg, the infusion of IPNA (2×10$^{-5}$, 2×10$^{-4}$, and
2×10^{-7} M) in kidneys from 7-week-old LH rats (n=4) induced a concentration-dependent renin release: from 147±29 before to 535±110, 1,086±145, and 1,260±218 ng Ang I/hr/min after the three concentrations of IPNA, respectively. The experiment was repeated with a perfusion solution free of calcium containing EGTA. A similar concentration-dependent renin release was observed but at higher values than in the presence of calcium: from 437±60 before to 761±99, 1,147±183, and 1,475±322 ng Ang I/hr/min after the three same concentrations of IPNA, respectively.

**Discussion**

The major finding of the present work is that the renin release of isolated perfused kidneys from young genetically hypertensive rats of the Lyon strain is not influenced by perfusion pressure as it is in kidneys of their normotensive controls. The present study was conducted to determine whether the baroreceptor control of renal renin could be abnormal in LH rats and thus lead to an inappropriate secretion that could contribute to the renin dependence of high blood pressure in that model.

Thus, we chose the isolated single pass perfused kidney preparation. Such an isolated organ is suitable to study the specific effects of perfusion pressure on renin release since it is free from sympathetic innervation and the perfusion medium is of known constant composition, and does not contain any stimulus or any component of the renin-angiotensin system. We designed a servocontrolled perfusion system to reach stable (±1 mm Hg) levels of RPP. With the perfused solution used, the sodium reabsorption rate was lower and the renal flow higher than in vivo. These findings are in accordance with those of others using similar preparations and are due to the relatively low oncotic pressure and viscosity of the perfusion solution compared with blood.

Kidneys were obtained from 7-week-old rats of the three Lyon strains, i.e., at an age preceding the full development of hypertension and major renal lesions in LH rats. Because we did not use a pulsatile perfusion system, in baseline conditions all the kidneys were...
perfused at the level of 90 mm Hg, which is approxi-
mately the mean arterial pressure in LN rats. In these
baseline conditions, LH kidneys, compared with both
LN and LL, exhibited significantly increased vascular
resistances and decreased GFR and urinary sodium
excretion. Such low GFR and urinary sodium excretion
are not observed in vivo. This may reflect the fact that,
in vivo, LH kidneys are perfused at higher levels than
LN or LL kidneys. If one considers that, as indicated by
the indirect SBP values recorded in the animals used,
the perfusion pressure of LH kidneys should establish
30 mm Hg above that of controls, the present work (see
Figure 2) demonstrates that the GFR and urinary
sodium excretion of LH kidneys perfused at 120 mm Hg
become identical to those of LN and LL kidneys per-
fused at 90 mm Hg. Therefore, according to Guyton's
hypothesis, these intrinsic renal alterations will allow
LH rats to maintain almost normal body fluids in spite
of an elevated blood pressure level.

According to Hofbauer et al.\textsuperscript{20} the study of perfusion
pressure involved stepwise reductions from 170 to 60
mm Hg, each level being maintained for 5 minutes since
our preliminary experiments showed that this duration
permitted the maximum renin response. Almost no
GFR and flow autoregulation could be observed in our
conditions. This reflects the large vasodilation of the
vessels of isolated kidneys perfused with artificial cell-
free solutions.\textsuperscript{21} Indeed, the infusion of hydralazine or the
use of calcium-free perfusion solutions did not
significantly increase the flow through isolated kidneys
(Liu, personal communication). When pressure was
elevated the fractional sodium reabsorption decreased
and the sodium excretion increased. LH kidneys dif-
fered from both LN and LL controls by lower perfusion
flow, GFR, and natriuresis. The blunted pressure-
natriuresis exhibited by LH kidneys appeared to be
mainly due to a lower filtered load but also to a slightly
higher sodium reabsorption rate.

Great care was taken to measure the renin release in
strictly controlled conditions involving the use of rat
angiotensinogen (biphenylcetomized rat plasma) and the
assessment of its excess by the decrease in renin
activity of Ang I production for each sample. This latter point
is important since renin release exhibited sevenfold
changes during the experiments. Using LN and LL
kidneys, a classic\textsuperscript{22} and reproducible pressure-depen-
dent renin release was observed that reached a plateau
at 85 mm Hg. Contrary to dog kidneys,\textsuperscript{23} renin release
increased as soon as the perfusion pressure decreased,
and no threshold pressure level was found. Surprisingly,
the renin release of LH kidneys appeared to be pressure-
independent. This data is in accordance with those of
Tobian et al.\textsuperscript{24,25} who used isolated kidneys of SHR or
Dahl salt-sensitive rats. In the present work, we could
rule out that the absence of renin release after decreases
in perfusion pressure was due to a lack of renin
storage in LH kidneys since these latter responded
dose-dependently to a β-adrenergic receptor stimula-
tion, a reduced extracellular calcium concentration, or
both. Because the sodium reabsorption rate remained
largely below that observed in vivo, an alteration in the
chloride-sensitive mechanisms of the macula densa\textsuperscript{26} is
probable in isolated kidney preparations. However, it is
likely to occur similarly in the kidneys of the three
strains. In addition, because the macula densa stimu-
lates renin release when it senses a decrease in chloride
delivery by the proximal tubule, any alteration in this
mechanism should have favored the renin release by the
LH kidney since its proximal tubular reabsorption of
NaCl is slightly higher than that of LN and LL controls.
Other probable explanations may involve structural
alterations (i.e., stiffness) of the renal vasculature of LH
rats, which are suggested by the lower responses of
perfusion flow and GFR to increases in pressure, or an
enhanced control of renin release by locally produced
angiotensin II\textsuperscript{27} since single pass--perfused isolated kid-
neys may be able to synthesize angiotensin II.\textsuperscript{28} A more
likely hypothesis is that the pressure independence of
renin release by LH kidneys could be a consequence of
a chronic sodium retention induced in these rats by their
increased synthesis of mineralocorticoids.\textsuperscript{29} De Rouffig-
nac et al.\textsuperscript{30} showed that sodium loading depleted a
promptly releasable store of renal renin and Fray\textsuperscript{31}
demonstrated that the renin release of sodium-loaded
kidneys was unresponsive to changes in pressure. A lack
of pressure influence on renin release in states of
sodium retention appears logical since it will contribute
to limiting the excess of body fluids. Obviously, further
experiments are needed to check this hypothesis and
determine the mechanisms involved.

Infusion of AH23848, a specific TXA\textsubscript{2}/PGH\textsubscript{2} recep-
tor antagonist, at a concentration of 4 x 10\textsuperscript{-6} M, which
blocks the effects of U46619,\textsuperscript{32} did not significantly
change the baseline characteristics of the kidneys of the
three strains. It also did not markedly alter the re-
sponses of LN and LL kidneys to changes in perfusion
pressure. On the contrary, it significantly improved the
pressure-induced increases in GFR and natriuresis in
LH kidneys. This data confirms those of Liu et al.,\textsuperscript{13} who
increased the perfusion pressure of LH kidneys by
means of norepinephrine infusions. Interestingly,
AH23848 significantly decreased the renin release by the
kidneys of the three strains. This result is in
accordance with those of Jackson et al.,\textsuperscript{33} demonstrating
that, in dogs, two different TXA\textsubscript{2} synthase inhibitors
lowered the renin release induced by decreases in RPP.
Conversely, Welch et al.\textsuperscript{34} reported that TXA\textsubscript{2} synthase
inhibition elevated the plasma renin activity in anesthe-
tized rats. However, it must be emphasized that in their
experiment, the doses of TXA\textsubscript{2} synthase inhibitors that
enhanced plasma renin were threefold higher than those
that blocked TXA\textsubscript{2} synthesis. Therefore, our data
taken together with those of Jackson et al.\textsuperscript{35} favor a
stimulating role on renin secretion for TXA\textsubscript{2}/PGH\textsubscript{2}.

In conclusion, the present work demonstrated in
servocontrolled single pass--perfused isolated kidneys
that the renin secretion of young LH rats is not stimu-
lated by decreases in perfusion pressure. Whatever
could be the underlying mechanisms, this finding sug-
gests that such a pressure independence of renin release
could represent an attempt to limit any further sodium
retention and blood pressure increase. In addition, it
was shown that the activation of intrarenal TXA\textsubscript{2}/PGH\textsubscript{2}
receptors enhances the renin release response to
changes in perfusion pressure. This latter observation
strengthens the potential pathogenetic role of the in-
creased renal synthesis of TXA\textsubscript{2} exhibited by kidneys of
genetically hypertensive rats of the Lyon strain.
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