Effect of Perindopril on Renal Medullary Hemodynamics in Genetically Hypertensive Rats

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**Background:** Early and chronic angiotensin converting enzyme (ACE) inhibition prevents hypertension and improves the pressure natriuresis in Lyon hypertensive (LH) rats. The effect of this treatment on the responses of renal medullary blood flow (MBF) to angiotensin II (Ang II) was studied.

**Methods:** In chronic experiments, Ang II (7.5 to 480 ng/kg, intravenous) was injected in 15-week-old anesthetized LH and normotensive (LL) control rats treated orally since weaning with perindopril (0.4 or 1.5 mg/kg/day) with or without pretreatment with indomethacin (5 mg/kg intravenous). In acute experiment, Ang II (30 to 480 ng/kg intravenous) was given in LH rats treated acutely with perindopril (1.5 mg/kg, intravenous bolus).

**Results:** Administration of Ang II induced dose-dependent decreases in MBF, which were greater in LH than in LL rats. In LL rats, the initial and short-lasting (<1 min) medullary vasoconstriction evoked by Ang II was followed by a long-lasting (>2 min) and dose-dependent medullary vasodilation, which was blunted in LH rats. Chronic perindopril treatment normalized the blood pressure level in LH rats and corrected their blunted medullary vasodilation, whereas the same treatment had no significant effect in LL rats. In chronically perindopril-treated LH rats, indomethacin decreased by 90% the medullary vasodilation induced by Ang II. Acute perindopril treatment did not modify the medullary responses to Ang II in LH rats.

**Conclusions:** Chronic ACE inhibition restores the vasodilator response of MBF to Ang II in LH rats. This effect, which is not observed after acute inhibition, is mainly mediated through the release of prostaglandins. Am J Hypertens 2006;19:617–622 © 2006 American Journal of Hypertension, Ltd.

**Key Words:** Hypertension, renin-angiotensin system, renal medullary circulation.

The kidney determines long-term blood pressure (BP) chiefly through the pressure-natriuresis function. However, the mechanisms underlying this function are not fully understood. It has been shown that, unlike total renal blood flow (RBF) and glomerular filtration rate, renal medullary blood flow (MBF) increases with arterial pressure elevations in volume-expanded rats. Recent studies indicate that changes in renal medullary hemodynamics alter renal interstitial hydrostatic pressure and that the medullary solute gradient play an important role in pressure-natriuresis. Furthermore it has been demonstrated that a primary reduction in MBF allows the development of hypertension, whereas an increase in MBF lowers hypertension. Thus MBF is believed to have a potent influence on renal sodium excretion and long-term BP regulation.

Lyon hypertensive rats (LH) are prone to retaining sodium because their pressure natriuresis is blunted and their hypertension is salt sensitive. The mechanisms involved in this sodium retention are unknown but may involve, at least in part, a lack of pressure dependency of MBF increases. Furthermore we observed that: 1) in response to angiotensin II (Ang II), LH rats exhibited an exaggerated medullary vasoconstriction and a reduced medullary vasodilatation compared with normotensive (LL) control rats, and 2) in LL rats, Ang II subtype 1 (AT1) receptor-mediated medullary vasodilator response is mainly caused by the release of prostaglandins (PG). Because hypertension can be fully prevented in LH rats and their pressure natriuresis markedly improved by chronic angiotensin converting enzyme (ACE) inhibition, we speculated that long-term treatment with perindopril might correct the altered MBF response to Ang II shown by LH rats. In addition, if a positive effect is observed in LH rats, it could be mediated through the release of PG. Therefore, the dose-related effect of perindopril on MBF was studied in LH and LL rats treated since weaning, with or without acute inhibition of PG by indomethacin. The effects of...
Ang II were also studied after acute administration of perindopril.

**Methods**

**Animals**

Male LH and LL rats were housed in controlled conditions (temperature, 21° ± 1°C; humidity, 60% ± 10%; lighting 8 to 20 h) and fed a standard diet (Elevage UAR, Villemoisson-sur-Orge, France) containing 0.3% sodium and tap water ad libitum. Studies were conducted in agreement with our institutional guidelines for animal care.

**Surgical Preparation**

Rats were anesthetized with inactin (thiobutabarbital sodium, 75 mg/kg, intraperitoneally, Research Biochemicals International, Natick, MA) and ketamine (25 mg/kg, intraperitoneally, Merial, Lyon, France) and placed on a heating blanket (model 50-6980, Harvard Apparatus, Edinbrige, KY) to maintain the rectal temperature at 37° ± 0.5°C. After a tracheotomy, the left jugular and femoral veins were cannulated for bolus injections and infusions, respectively. The left carotid artery was cannulated to record the mean BP through a pressure transducer (model P23ID, Statham Instrument Division, Gould Inc., Cleveland, OH). To replace fluids lost during surgery, a 5% bovine albumin (fraction V, Sigma Chemical, St. Louis, MO) in 0.9% NaCl solution was infused (0.33 mL/kg/min) for 30 min and then replaced by a 1% bovine albumin infused at the same rate during the overall experiment. Through a midline abdominal incision the left renal artery was carefully dissected and an ultrasonic transit-time flow probe (1RB, Transonic Systems Inc., Ithaca, NY) was placed to record total RBF using a transit-time flowmeter (model T106, Transonic Systems Inc.). The left kidney was freed from its surrounding tissue and immobilized in a plastic cup to avoid respiration-induced movements. A needle laser Doppler flow probe (400 μm diameter, Model 411, Perimed, Järfalla, Sweden) was inserted perpendicularly into the middle part of the left kidney through a hole made in the capsule using a 25-gauge needle and advanced to a depth of 5 mm in the medulla. This was made using a stereotaxic apparatus (model 900, David Kopf Instruments, Tujunga, CA). The probe was connected to a flowmeter (Laser Doppler System, Periflux 4001 Master, Perimed) for measurement of MBF and calibrated before the experiment using a motility standard (PF 1001, Perimed). Pulsatile arterial pressure, total RBF, and MBF were continuously monitored using a computerized recording system (Lab View Software, National Instruments, Austin, TX).

**Experimental Protocols**

**Injections of Ang II in Control and Chronically Perindopril-Treated LL and LH Rats**  
From 3 to 15 weeks of age, LH rats were orally treated by perindopril (Servier Laboratories, Neuilly-sur-Seine, France) at the dose of 0.4 (P0.4, n = 8) or 1.5 (P1.5, n = 9) mg/kg/day given through the drinking water. The highest dose of perindopril was also given in LL rats (n = 7). Perindopril concentration was adjusted weekly to the water intake and body weight of rats. Untreated LH (n = 9) and LL (n = 8) rats received water only and served as controls.

After surgical preparation, a 50-min period allowed for stabilization. Parameters were recorded for 10 min in baseline conditions in control and perindopril-treated rats. Then, intravenous bolus (7.5, 30, 120, and 480 ng/kg) of Ang II (Sigma Chemical) were given. Two consecutive injections were separated by a period of 10 min to allow a full recovery of parameters. For each dose of Ang II, the response was calculated as the percentage change from the baseline values immediately before injection.

**Injections of Ang II After PG Inhibition in Chronically Perindopril-Treated LH Rats**

In LH rats treated from 3 to 15 weeks of age by the highest dose of perindopril (1.5 mg/kg/day, orally), 25 min after surgical preparation, baseline values of parameters were recorded for 10 min. The rats then received an intravenous injection of indomethacin (Sigma Chemical) at a dose of 5 mg/kg. In this group, 20 min after pretreatment, the parameters were recorded for 10 min, and injections of Ang II (7.5 to 480 ng/kg) were then performed and the responses calculated as described above.

**Injection of Ang II in Acutely Perindopril-Treated LH Rats**

Fifteen-week-old LH rats were used. After surgical preparation, 50 min were allowed for stabilization. Parameters were recorded for 10 min in baseline conditions, and intravenous boluses (30 to 480 ng/kg) of Ang II were then given as described above. The animals then received an intravenous administration of perindopril (1.5 mg/kg), 40 min after the perindopril injection, the parameters were recorded for 10 min, and the doses of Ang II (30 to 480 ng/kg) were repeated. The responses were calculated as described above. The dose of perindopril was chosen to abolish the vasopressor effects of angiotensin I (Ang I, 750 ng/kg), which increased mean BP by 37% ± 5% and lowered RBF by 82% ± 5%.

**Statistical Analysis**

Values are means ± SEM. The baseline differences between groups were analyzed by the Student t test for unpaired data. The intergroup differences in the dose–response curves of Ang II and its dose-related effects within groups were analyzed using analysis of variance (ANOVA) for repeated measures. The pairwise contrasts of mean at each dose were also made by the step-down Holm-Bonferroni procedures.11 The differences before and after acute perindopril treatment were determined by Wilcoxon test for paired data. A difference was considered to be significant at values of P < 0.05.
Table 1. Blood pressure and renal parameters obtained in 15-week-old male anesthetized Lyon hypertensive (LH) and normotensive (LL) rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean BP (mm Hg)</th>
<th>RBF (mL/min/g kw)</th>
<th>RVR (mm Hg/mL/min/g kw)</th>
<th>MBF (PU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>9</td>
<td>161 ± 5*</td>
<td>5.8 ± 0.4</td>
<td>29.1 ± 2.4*</td>
<td>258 ± 23</td>
</tr>
<tr>
<td>LLP0.4</td>
<td>8</td>
<td>131 ± 3†</td>
<td>6.2 ± 0.3</td>
<td>19.9 ± 1.4†</td>
<td>275 ± 23</td>
</tr>
<tr>
<td>LLP1.5</td>
<td>9</td>
<td>116 ± 5‡‡</td>
<td>7.2 ± 1.1§</td>
<td>15.8 ± 1.4†</td>
<td>287 ± 21</td>
</tr>
<tr>
<td>LL</td>
<td>8</td>
<td>121 ± 2</td>
<td>6.9 ± 0.5</td>
<td>18.1 ± 1.2</td>
<td>270 ± 23</td>
</tr>
<tr>
<td>LLP1.5</td>
<td>7</td>
<td>112 ± 4</td>
<td>7.1 ± 0.2</td>
<td>15.8 ± 0.7</td>
<td>240 ± 20</td>
</tr>
</tbody>
</table>

BP = blood pressure; KW = left kidney weight; MBF = medullary blood flow; n = number of rats; RBF = total renal blood flow; RVR = renal vascular resistance.

Data are mean ± SEM. Perindopril was given orally from 3 to 15 weeks of age at the dose of 0.4 (P0.4) or 1.5 (P1.5) mg/kg/day.

* P < .001 v LL.
† P < .001 v untreated LH rats.
‡ P < .05 v LH P0.4.
§ P < .01.

Results

Injections of Ang II in Control and Chronically Perindopril-Treated LL and LH Rats

Before Ang II injections (Table 1), LH differed from LL rats by a higher mean BP and lower RBF leading to an elevated RVR. Chronic treatment with perindopril dose-dependently decreased mean BP and RVR, and increased total RBF in LH rats. At the dose of 1.5 mg/kg/day, perindopril normalized the mean BP and renal hemodynamics of LH rats, whereas it had no significant effect in LL rats.

As shown in Fig. 1, Ang II dose-dependently increased mean BP and decreased RBF in both LL and LH rats. In LL rats, the response of MBF was biphasic and dose-related, with an initial rapid and short-lasting (<1 min) decrease (vasoconstrictor component) followed by a marked and longer-lasting (>2 min) increase (vasodilator component). In LH rats, the initial vasoconstrictor component was more pronounced, whereas the vasodilator component was reduced.

Figure 2 shows that mean BP increased dose-dependently and RBF decreased after Ang II injections in both untreated strains. Interestingly, the biphasic response of MBF was dose-related in both LL and LH rats. Compared with LL rats, the increase in MBF (vasodilator component) was reduced in LH rats, whereas the decrease in MBF (vasoconstrictor component) was more pronounced in LH than in LL controls.

Figure 3 shows that in LH rats, the two doses of perindopril did not modify the increase in mean BP evoked by Ang II. However, the highest dose of perindopril significantly amplified the decrease of RBF. Perindopril dose-dependently increased the vasodilator component of MBF response to Ang II in LH rats, whereas it did not significantly change the vasoconstrictor component. In LL rats, perindopril did not modify the response of mean BP evoked by Ang II but significantly amplified the response of RBF; neither the vasodilator component nor the vasoconstrictor component was significantly modified by perindopril.

Injections of Ang II After PG Inhibition in Chronically Perindopril-Treated LH Rats

Before Ang II injections, indomethacin did not significantly modify mean BP (125 ± 4 vs 117 ± 4 mm Hg), but significantly decreased total RBF (from 7.5 ± 0.5 to 6.5 ± 0.5 mL/min/g left kidney weight [kw], P < .05) and MBF (from 185 ± 28 to 142 ± 28 PU, P < .05). As shown in Figure 4,
in response to increasing doses of Ang II the response of mean BP was unchanged. However the decrease in both total RBF and MBF was enhanced by the pretreatment with indomethacin. Interestingly indomethacin attenuated the increase in MBF by about 90% from the low doses.

Injections of Ang II in Acutely Perindopril-Treated LH Rats

Acute treatment with perindopril in LH rats did not significantly modify mean BP (164 ± 3 vs 162 ± 3 mm Hg), RBF (5.5 ± 0.3 vs 6.0 ± 0.4 mL/min/g) and MBF (171 ± 13 vs 183 ± 16 PU). As shown in Figure 5, in response to increasing doses of Ang II, acute pretreatment with perindopril did not modify the increase in mean BP, whereas it significantly amplified the decrease of RBF. Neither the vasodilator component nor the vasoconstrictor component of MBF responses to Ang II was modified by acute perindopril administration.

Discussion

The present work shows that: 1) early and chronic treatment with perindopril in LH rats, which prevents the hypertension, improves the blunted vasodilator response of MBF to Ang II, and this effect is not observed with acute administration of perindopril; 2) the inhibition of PG production markedly counteracts the beneficial effects of ACE inhibitor on renal medullary circulation.

Injections of Ang II produced a biphasic MBF response in Lyon rats. This response was not related to the manner by which Ang II was administered and did not occur after bolus injections of phenylephrine, despite a similar increase in BP and decrease in RBF. A biphasic MBF response to Ang II injection was also reported in anesthetized rabbits. In addition, the increase in MBF has been shown to occur with Ang II infusion even if the increase in BP was prevented by an aortic clamp. A greater total

FIG. 2. Percentage changes in mean BP, total RBF, and MBF in response to Ang II injections in 15 week-old LL and LH rats. *P < .05 and ***P < .001 LH v LL rats. Abbreviations in Fig. 1.

FIG. 3. Percentage changes in mean BP, total RBF, and MBF in response to Ang II injections in controls (C) and rats treated orally from 3 to 15 weeks of age with perindopril at the dose of 0.4 (P0.4) or 1.5 (P1.5) mg/kg/day. *P < .05, **P < .01, and ***P < .001 P1.5 v controls; †P < .05 P 0.4 v controls. Abbreviations as in Fig 1.
renal hemodynamic and tubular response to Ang II was previously reported in LH rats. In the present work, LH rats exhibited an increased vasoconstrictor and a blunted vasodilator response of MBF compared with LL rats. This indicates that the exaggerated response to the vasoconstrictor effect of Ang II in LH kidney also concerns the medullary vascular bed. Accordingly it has been shown that the renal medullary circulation of SHR is more sensitive to the vasoconstrictor effect of Ang II compared with that of their normotensive controls. However, in contrast to LH rats, a medullary vasodilation was not observed in SHR after the vasoconstriction induced by Ang II.

Perindopril (1.5 mg/kg/day, orally) did not significantly modify BP in LL rats but normalized the high BP level in LH rats. In both chronically and acutely perindopril-treated rats, the total RBF response to Ang II was enhanced. The renal vasoconstriction seen after Ang II injections might have resulted from the autoregulatory phenomena that occur in response to acute systemic BP elevation. However this is unlikely to have been the only mechanism responsible, as the doses of Ang II that had no effect on mean BP have been shown to reduce RBF.

**FIG. 4.** Percentage changes in mean BP, RBF, and MBF in response to Ang II injections in LH rats chronically treated orally with perindopril alone (1.5 mg/kg/day, LHP1.5) or combined to an acute pretreatment with indomethacin (LHP1.5 + Indo). *P < .05, **P < .01, and ***P < .001 LHP1.5 + Indo v LHP1.5.

**FIG. 5.** Percentage changes in mean BP, total RBF, and MBF in response to Ang II injections in LH rats before (Before) and 40 min after (After) administration of perindopril (1.5 mg/kg, intravenously). *P < .05 after v before perindopril.
underlying reason for enhanced RBF response to Ang II seen in perindopril-treated rats is unknown. However, an upregulation of AT1 receptors was reported after the inhibition of endogenous Ang II formation. Interestingly, after chronic perindopril treatment, the vasodilator response of MBF to Ang II in LH rats was restored to the level observed in LL rats. The similar effect is also observed in SHR after long-term ACE inhibition with enalapril. To determine whether acute ACE inhibition alters the renal response to Ang II in LH rats, an additional experiment was realized with the dose of perindopril that abolished the pressor response to a high dose of Ang I. The finding that acute perindopril treatment had no effect suggests that the restoration of the MBF response in LH rats is caused mainly by a chronic mechanism or mechanisms rather than an by immediate suppression of the circulating Ang II or an increased bradykinin formation.

In LL rats, both the initial decrease and delayed increase in MBF evoked by Ang II were AT1 receptor dependent. The vasodilator component induced by low doses of Ang II involved mainly the release of PG, whereas high doses was related to nitric oxide and kinin-dependent components. No published information is available concerning the medullary production of these vasodilators in LH rats. With regard to the ACE levels in the renal medulla, an abundant expression of ACE mRNA and a high ACE activity were reported in this region. In addition ACE inhibition has been shown to increase the release of vasodilatory PG. In the present work we examined whether the chronic action of perindopril on MBF could be related to PG. Because perindopril had no effect in LL rats, only LH rats were studied. Under baseline conditions indomethacin did not modify the BP levels in chronically perindopril-treated LH rats but did significantly decrease their total RBF and MBF. This decrease was about threefold greater than that previously observed in untreated rats, suggesting that PG were increased and actively contributed to the renal whole and medullary perfusion. This finding also demonstrates that cyclooxygenase inhibitor counteracts the beneficial renal effect of ACE inhibition. In response to Ang II, indomethacin significantly attenuated the increased vasodilator MBF response shown by perindopril-treated LH rats and enhanced their vasoconstrictor response. These results were consistent with an early study showing that the inhibition of PG synthesis resulted in an increased renal sensitivity to Ang II and provide evidence that the PG production also actively contributed to the medullary protection against vasoconstriction. The mechanism underlying the PG production after long-term ACE inhibition in LH rats remains to be investigated.

In conclusion, the present work demonstrates that the altered vasodilator MBF response to Ang II shown by LH rats is restored by an early and chronic ACE inhibition and this effect, which may contribute to lowering BP, mainly results from the release of PG. In addition, the study shows that inhibition of PG production reduces the renal medullary protection conferred by chronic ACE inhibition.

References