Renal Responses to AT$_1$ Blockade in Angiotensin II-Induced Hypertensive Rats

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Abstract. Previous studies have shown that uninephrectomized rats infused chronically with low doses of angiotensin II (Ang II) develop progressive hypertension that is prevented by co-administration of losartan in the drinking water. The present study was performed to contrast the effects of chronic and acute losartan treatment in reversing the Ang II-mediated actions on arterial pressure and renal function. Ang II was infused subcutaneously via osmotic minipumps (40 ng/min) for 13 days in two groups (N = 10 and N = 6); one group also received losartan in the drinking water (30 mg/kg-day) throughout this period. Untreated rats (N = 6) and rats (N = 6) receiving only losartan served as control groups. Ang II-infused rats had higher mean arterial pressures (153 ± 7 versus 107 ± 3 mm Hg) and lower GFR (0.7 ± 0.04 versus 0.98 ± 0.06 mL/min/g) than Ang II-infused rats receiving losartan chronically. The Ang II-infused rats responded to acute doses of losartan (10 mg/kg) with progressive reductions in arterial pressure and significant increases in cortical blood flow (34 ± 12% increase), renal plasma flow, GFR, and sodium excretion; however, the increases in renal blood flow and GFR were not sustained as systemic arterial pressure decreased. Because Ang II-infused rats receiving losartan chronically still exhibited decreases in RBF in response to a bolus dose of Ang II, further studies evaluated the effects of acute losartan treatment in rats treated chronically with losartan. Although arterial pressure decreased only slightly, demonstrating adequate systemic vascular blockade, there were still substantial and sustained increases in renal plasma flow, cortical blood flow (20 ± 4% increase), GFR, and sodium excretion. In summary, the modest responses to acute losartan in Ang II-infused rats indicate that chronic Ang II infusions lead to alterations in renal function that are only partially reversible by acute losartan treatment. In contrast, chronic treatment with losartan prevents the Ang II-induced decrease in GFR. The renal responses to acute losartan in the Ang II-infused rats treated chronically with losartan suggest that substantive intrarenal actions of Ang II can be maintained even when the systemic vascular AT$_1$ receptors are effectively blocked. (J Am Soc Nephrol 8: 535–542, 1997)

The renin-angiotensin system is known to contribute to elevated arterial pressure and associated reductions in renal functions in various forms of hypertension (1–7). In the two-kidney, one-clip (2K1C) model of hypertension, it has been shown that the nonclipped kidney responds to angiotensin converting enzyme inhibitors and angiotensin II (Ang II) receptor antagonists; it also exhibits increased intrarenal Ang II levels even though it has very low tissue renin levels (1,4,7–11). The elevated circulating Ang II concentrations lead to increases in intrarenal Ang II levels and reduce function of the nonclipped kidney (7,8,12). It has also been shown that chronic infusions of Ang II doses that are initially subpressor can enhance pressor responses to Ang II and lead to a slowly developing hypertension and elevations of intrarenal Ang II levels similar to that observed in 2K1C Goldblatt hypertension (8,12–17). Because renal renin content and renin gene mRNA levels are suppressed, these results provide further support to the hypothesis that renal function in this model is also influenced by circulating Ang II causing enhanced intrarenal Ang II levels (8,12,17).

Recent studies have demonstrated that concomitant chronic AT$_1$ receptor blockade by the addition of losartan to the drinking water prevents the development of hypertension and reduces the degree of renal injury in 2K1C Goldblatt hypertensive rats and in Ang II-infused rats (11,14). In particular, chronic AT$_1$ blockade prevented the augmentation of intrarenal Ang II levels caused by chronic Ang II infusions (14). These data suggest that chronic losartan treatment attenuates the progressive renal effects of Ang II infusions. Previous studies have suggested that acute losartan treatment is not as effective as chronic losartan treatment in normalizing renal function in 2K1C Goldblatt hypertensive rats or spontaneously hypertensive rats (5,11,18,19). Presumably, this is due to the slowly developing renal structural alterations associated with the development of hypertension (11,14). To evaluate this issue in Ang II-induced hypertension, renal function in Ang II-infused rats receiving chronic losartan treatment was compared with renal function in Ang II-infused rats after acute treatment with losartan. Accordingly, the primary aims of the present study were to compare renal function in Ang II-infused rats with that in Ang II rats treated chronically with losartan and to determine differences between the effects of acute and chronic losartan...
treatment on renal function in Ang II-infused rats. In both groups, the effectiveness of blockade was tested by administering bolus doses of 50 ng of Ang II. It was noted that while the arterial pressure effects of Ang II were adequately blocked in the rats treated chronically with losartan, Ang II still elicited a decrease in cortical renal blood flow suggesting differential effects on the systemic vasculature and the kidney. Therefore, losartan was also administered acutely to the rats treated chronically with losartan. Interestingly, we found that while rats treated chronically with losartan had only a very slight decrease in mean arterial pressure during acute losartan infusion, they still exhibited marked increases in renal function. This allowed assessment of renal hemodynamic and excretory responses to AT1 receptor blockade without the confounding effects caused by associated reductions in arterial pressure.

Materials and Methods

General Procedures

Male Sprague Dawley rats (Charles River, Wilmington, MA), body weight 170–230 g, were housed in wire cages in rooms with light and temperature control. They were fed normal rat chow and assigned randomly into four groups. All rats were anesthetized with sodium pentobarbital (50 mg/kg ip) and subjected to a right nephrectomy via a flank incision. Osmotic minipumps (Model 2002, Alza Corp, Palo Alto, CA) containing Ang II (Sigma Chemical Co., St. Louis, MO) at concentrations sufficient to allow an infusion rate of 40 ng/min were implanted subcutaneously in rats from groups I (N = 10) and II (N = 6). Previous studies have shown that this dose of Ang II leads to a slowly developing hypertension over the course of 8–13 days (8,14–16). Rats in groups III (N = 6) and IV (N = 6) received only a right nephrectomy. After recovery, rats in groups II and IV were given losartan (Du Pont-Merck Pharmaceutical Co, Wilmington, DE) in the drinking water (30 mg/kg.day), whereas groups I and III remained untreated. Rats were housed in the vivarium for 13 days before the acute clearance experiments.

Experimental Protocol

Thirteen days after nephrectomy, rats were prepared for acute clearance experiments as previously described (8,18,20). After induction of anesthesia with sodium pentobarbital (50 mg/kg ip), rats were put on a temperature-controlled table, and body temperature was maintained at 37–37.5°C. A tracheotomy was done and a short PE-240 tube was inserted to maintain a patent airway. The left jugular vein was isolated and catheterized with a PE-50 catheter, for fluid infusion, and a PE-10 catheter, for administration of sodium pentobarbital, as needed to maintain an appropriate level of anesthesia. The left femoral artery was exposed through the left inguinal area, and a PE-50 catheter was inserted and connected to a Grass Polygraph (Grass Instrument Co., Quincy, MA, USA) via a Statham pressure transducer for arterial pressure monitoring. A left flank incision was made, and the kidney was isolated from the surrounding tissue and placed in a plastic cup to keep it stable. The tip of a laser-Doppler flow probe (Med Pacific, Seattle, WA) was placed close to the surface of the kidney for measurement of relative changes of cortical blood flow. The laser-Doppler flow technology allows dynamic assessment of relative changes in renal blood flow (20). A PE-10 tube was inserted into the ureter for urine collections.

During surgery, an isotonic saline solution containing albumin (6 g/dL) was infused at a rate of 20 μL/min. After surgery, an isotonic saline solution containing albumin (1 g/dL), p-aminohippurate sodium (PAH) (1.5 g/dL) and inulin (Intest) (2 g/dL) was infused at the same infusion rate. After the surgical procedure, a 1-hr equilibration period was allowed before starting urine collections and control clearance periods. Urine samples were collected every 30 min and blood samples were collected every 30 min during and after the losartan treatment. Two blood samples were collected to calculate inulin and PAH clearance and assess the renal functional responses to acute losartan treatment. Adequacy of AT1 receptor blockade was tested by giving bolus doses of Ang II (50 ng) and noting the responses in arterial pressure and cortical renal blood flow. The acute dose of losartan (10 mg/kg body weight) effectively blocked both pressor and renal blood flow responses to Ang II bolus. In contrast, the Ang II-infused rats treated chronically with losartan still exhibited a decrease in cortical blood flow with the bolus dose of Ang II although the pressor responses were blocked. These observations provide the rationale for the studies in which acute losartan was given to rats treated chronically with losartan.

Analytical Procedures

Blood and urine samples were analyzed for inulin, PAH, sodium, and potassium concentrations. Inulin and PAH concentrations were measured colorimetrically. Sodium and potassium concentrations were determined by flame photometry.

Calculations and Statistical Analyses

GFR was calculated from urine inulin and plasma inulin concentrations and urine flow. PAH clearance was used as an index of RPF. Urine sodium concentration, and urine flow were used to calculate the sodium excretion rate, and fractional sodium excretion was calculated from the ratio of urine and plasma sodium concentrations divided by urine and plasma inulin concentrations. Fractional potassium excretion was calculated from the ratio of urine and plasma potassium concentrations divided by urine and plasma inulin concentrations. Results are expressed as mean ± SEM. Data comparing renal function among groups were analyzed by one-way ANOVA, and the responses to acute losartan treatment were analyzed by repeated measures ANOVA. Values exceeding the 95% critical limits (P < 0.05%) are considered to be statistically significant.

Results

Influences of Chronic Losartan Treatment on Arterial Pressure and Renal Function

As previously shown (8,14–16), infusion of Ang II for 13 days led to significant increases in arterial pressure (153 ± 7 mm Hg). As shown in Figure 1A, rats infused with Ang II but treated chronically with losartan did not develop hypertension (107 ± 3 mm Hg) and had arterial pressures similar to the control rats treated with losartan. Control rats treated chronically with losartan had lower arterial pressures from that observed in nontreated control rats (106 ± 4 versus 121 ± 2 mm Hg, P < 0.05). Baseline measurements of GFR are shown in Figure 1B and indicate that the Ang II-infused rats had significantly lower GFR values compared with control rats. Chronic losartan treatment prevented the decrease of GFR caused by...
Figure 1. (A) Comparison of basal mean arterial pressures (MAP) and (B) glomerular filtration rate (GFR) before acute losartan treatment in four groups of rats (N = 6 in each group). Ang II + LOS and control + LOS groups were given losartan (30 mg/kg-day) in drinking water for 12–13 days. Values are mean ± SE. *P < 0.05 compared with control group.

Ang II infusions (0.98 ± 0.06 versus 0.7 ± 0.04 mL/min·g, P < 0.05) and maintained GFR at values similar to those found in control rats. GFR values in losartan-treated rats were also similar to those found in control rats indicating that chronic losartan treatment did not significantly influence GFR in control rats. Estimated renal plasma flow (RPF) values based on PAH clearance suggested that RPF was higher in the Ang II-infused rats receiving chronic losartan treatment than in the
Ang II-infused rats (4.5 ± 0.4 versus 3.6 ± 0.4 mL/min·g), but the differences are not statistically significant.

Effects of Acute Losartan Treatment on Arterial Pressure

As shown in Figure 2, acute losartan treatment led to progressive decreases in MAP in the Ang II-infused hypertensive rats over the course of 90 min but did not reduce the arterial pressures down to values seen in rats treated chronically with losartan within the time frame of the experiments. MAP was maintained during the infusion period but then fell progressively in response to acute losartan treatment from 153 ± 7 to 119 ± 5 mm Hg by the end of 90 min. Time control rats infused with Ang II (N = 4) but not given acute losartan maintained their MAP during this period (163 ± 16 to 167 ± 18 mm Hg). Acute losartan treatment also decreased MAP in control rats but to a lesser extent than in Ang II-infused rats (11 ± 2 versus 34 ± 5 mm Hg). In Ang II-infused rats receiving losartan chronically, MAP decreased very slightly from 107 ± 3 mm Hg to 102 ± 5 mm Hg indicating the systemic effectiveness of chronic AT₁ receptor blockade. Control rats treated with losartan alone also had only a small decrease in arterial pressure after acute losartan (5 ± 4 mm Hg).

Effects of Acute Losartan Treatment on Renal Hemodynamic Function

As shown in Figure 3, PAH clearance and cortical blood flow increased significantly in response to acute infusion of losartan in all groups except control rats receiving losartan

Figure 2. Comparison of MAP after acute losartan treatment in four groups of rats (N = 6 in each group). Losartan (10 mg/kg) was given over a period of 30 minutes. ■, Ang II; □, Ang II + LOS; ▲, control; △, control + LOS; Values are mean ± SEM. *P < 0.05 compared with control time.

Figure 3. (A) Comparison of PAH clearance and (B) relative changes in cortical blood flow after acute losartan treatment in four groups of rats (N = 6 in each group). ■, Ang II; □, Ang II + LOS; ▲, control; △, control + LOS; Values are mean ± SEM. *P < 0.05 compared with control time.

chronically. In the Ang II-infused rats, PAH clearance increased from 3.6 ± 0.4 to 4.9 ± 0.7 mL/min·g and cortical blood flow (CBF) increased by 34 ± 12% during the infusion period before arterial pressure began to fall. However, these increases in plasma flow and CBF waned in subsequent clearance periods coincident with the decreases in arterial pressure. Interestingly, the Ang II-infused rats receiving losartan chronically also exhibited substantial increases in plasma flow and CBF after the acute losartan dose reaching maximum PAH clearance levels of 6.1 ± 0.9 mL/min·g and a 20 ± 4% increase in CBF. This response was relatively well sustained, which
could be because the arterial pressure was also well maintained in this group. The control group also exhibited an increase in RPF that increased to $5.1 \pm 0.3$ mL/min/g; CBF increased by $34 \pm 5\%$. In contrast to other groups, the control rats receiving losartan chronically did not exhibit significant increases in RPF or CBF $(3.7 \pm 0.3$ to $3.9 \pm 0.5$ mL/min/g, $P > 0.05$, and $9.7 \pm 3.4\%$ change, $P > 0.05$). Time control rats infused with Ang II but not given acute losartan did not have perceptible changes in RPF $(2.9 \pm 0.5$ versus $3.0 \pm 0.5$ mL/min/g) or CBF (0 to 7 ± 9%) during this period.

Effects of Acute Losartan Treatment on GFR
As shown in Figure 4, GFR in the Ang II-infused rats increased slightly during acute losartan infusion period; however, the increase was not sustained, and GFR returned to control levels as arterial pressure fell. The control rats also exhibited a slight increase in GFR by 60 min $(1.04 \pm 0.07$ to $1.20 \pm 0.05$ mL/min/g, $P < 0.05$). The Ang II rats receiving losartan chronically slowly developed a small increase in GFR, which achieved statistical significance 60 min after infusion $(0.98 \pm 0.06$ to $1.19 \pm 0.12$ mL/min/g, $P < 0.05$). GFR in control rats receiving losartan chronically did not respond to acute losartan treatment. Time control rats infused with Ang II but not given acute losartan maintained their GFR $(0.47 \pm 0.04$ to $0.52 \pm 0.07$ mL/min/g) during this period.

Effects of Acute Losartan Treatment on Excretory Function
As shown in Figure 5, all groups showed significant increases in sodium excretion in response to acute losartan treat-

Figure 4. Comparison of glomerular filtration rate (GFR) in response to acute losartan treatment in four groups of rats ($N = 6$ in each group). ■, Ang II; ＄, Ang II + LOS; ▲, control; △, control + LOS; Values are mean ± SEM. *$P < 0.05$ versus control time. **$P < 0.05$ compared with control group.

Figure 5. (A) Comparison of sodium excretion and (B) fractional sodium excretion after acute losartan treatment in four groups of rats ($N = 6$ in each group). ■, Ang II; ＄, Ang II + LOS; ▲, control; △, control + LOS; Values are mean ± SEM. *$P < 0.05$ compared with control time.
Discussion

The initial objectives of this study were to evaluate the ability of chronic AT_1 receptor blockade to prevent the renal functional alterations that occur during Ang II-induced hypertension and to compare the renal effects of chronic blockade with the effects of acute treatment with losartan. It was anticipated that acute losartan administration to Ang II-infused hypertensive rats would reverse the existing functional influences of the elevated intrarenal Ang II levels but not the changes in renal function caused by structural alterations occurring as a consequence of the chronic Ang II infusions (14). In agreement with previous findings (5,11,14,18,19), it was found that chronic treatment with losartan prevented the development of hypertension. Arterial pressures in the Ang II-infused rats treated with losartan remained at normotensive levels and were similar to those measured in control rats receiving chronic losartan. In addition, the reductions in GFR caused by long-term Ang II infusion were ameliorated by chronic losartan treatment. These data are consistent with previous findings showing that the Ang II-induced enhancement of intrarenal Ang II is also prevented by losartan (14).

Acute losartan treatment to the Ang II-infused rats reduced systemic arterial pressure and increased RPF and CBF. GFR was also increased during the losartan infusion period but there was not a sustained increase in GFR in the Ang II-infused rats in the face of slowly declining arterial pressure. GFR in the Ang II-infused rats treated acutely with losartan did not reach GFR values in Ang II-infused rats treated chronically with losartan. These results reflect the progressive action of chronic increases in circulating and renal Ang II to elicit intrarenal structural changes not rapidly reversible by acute Ang II blockade as has been noted to occur in hypertensive models (5,11,14,18,21). According to a recent study of short- and long-term effects of losartan in hypertensive rats by Kline and Liu (5), long-term losartan treatment shifted the pressure natriuresis curve to the left whereas acute losartan treatment did not cause a similar effect.

As noted, acute losartan treatment to the Ang II-infused rats also caused progressive decreases in arterial pressure through-out the course of the clearance measurements. Interestingly, there was a temporal difference in the effects of losartan on arterial pressure, which were delayed and developed slowly while the maximum effects on CBF were observed within the first period. This observation suggests differences between the systemic vascular and renal AT_1 receptors but could also be caused by a resetting of baroreflexes that might elicit counteracting responses on systemic arterial pressure. Nevertheless, the decrease in arterial pressure could have partially attenuated the direct renal effects of losartan to increase blood flow, GFR, and sodium excretion. Such depressor effects of Ang II blockade or ACE inhibition occur often in angiotensin-dependent models of hypertension and have led to difficulties in delineating the direct renal effects from the indirect effects of the reduced arterial pressure and of the compensatory responses to the pressure reductions (8,18,22,23). In 2K1C Goldblatt hypertensive rats, acute losartan treatment has been shown to exert such profound vasodepressor effects that GFR and sodium excretion may decrease (18,24). When the AT_1 receptor blockade is confined primarily to the kidney, the effects to increase sodium excretion are greater (24).

Several previous studies have suggested that lower doses of receptor blockers are needed to block Ang II-dependent influences on the systemic vasculature than on the renal vasculature (25,26). In this study, adequacy of AT_1 blockade was evaluated by administering bolus doses of Ang II or infusing losartan and comparing the systemic pressure and renal functional responses. It was noted that when losartan was administered acutely to the Ang II-infused rats treated chronically with losartan, there were only minor effects on arterial pressure, but there were marked increases in RPF, CBF, and sodium excretion. The marked increases in fractional sodium excretion in response to acute losartan suggest that the higher doses of losartan may be blocking the tubular AT_1 receptors as well as the vascular AT_1 receptors (27,29). Because systemic arterial pressure fell only slightly in this group of rats, the direct renal vasodilator and natriuretic effects of AT_1 blockade could be manifested fully without the counteracting actions of decreases in arterial pressure. These data indicate that, even when the systemic vascular AT_1 receptors are effectively blocked, renal AT_1 receptors may still be exerting an influence.

In this model of Ang II-induced hypertension, systemic arterial pressure increased progressively over the course of the 13–14 days of infusion. Although it has been reported that down regulation of vascular AT_1 receptors occurs during Ang II infusions (27–29), more recent studies have suggested that preglomerular vascular AT_1 receptors are not significantly reduced by 7 days of Ang II infusions at both pressor and subpressor doses (30). Some studies have also indicated that the increase of systemic arterial pressure is partly caused by sympathetic activation after chronic Ang II infusion (15,16,31). Regardless of the detailed mechanism responsible for the slowly developing increases in arterial pressure, chronic treatment with losartan in the drinking water of the Ang II-infused rats fully prevented the development of hypertension in the Ang II-infused rats. Nevertheless, the renal hemodynamic and sodium excretory responses observed in Ang II-infused...
rats receiving losartan chronically indicate that vascular and tubular losartan-sensitive Ang II receptors continued to exert effects on renal vascular resistance, GFR, and sodium excretion. This differential sensitivity of systemic and renal systems further supports recent studies indicating the presence of losartan-sensitive Ang II receptors in the kidney that have a lower affinity to losartan and are not down regulated by circulating Ang II (27,29,32).

Several studies have demonstrated that, in contrast to their effects on vascular Ang II receptors, Ang II infusions lead to up regulation of tubular Ang II receptors (27,29). More recently, it was shown specifically that AT₁ receptors in proximal tubule cells are increased by Ang II (29). Presumably, these receptors were not completely blocked by the losartan levels achieved during chronic losartan treatment, thus explaining the natriuretic responses to the acute losartan dose observed in this present study. Furthermore, Wang and Du (33) recently reported that rats treated with losartan and a low salt diet to increase endogenous Ang II levels manifested enhanced renal AT₁A mRNA expression, but not adrenal AT₁ mRNA expression. Thus, losartan did not prevent the Ang II-mediated increases in intrarenal AT₁A mRNA expression suggesting possible increases in intrarenal AT₁ receptors in rats infused with Ang II and treated chronically with losartan. Such receptors could continue to exert intrarenal actions even when the systemic vascular actions are effectively blocked by lower doses of losartan.

In summary, the results indicate that Ang II-induced hypertension is associated with a reduced GFR, which can not be fully restored by acute treatment with losartan. In contrast, chronic treatment with losartan prevents the decreases in GFR and RPF along with the increases in arterial pressure. However, chronic losartan treatment sufficient to achieve adequate systemic vascular blockade does not completely block the intrarenal responsiveness to Ang II and acute doses of losartan elicit further increases in RBF, GFR, and sodium excretion. Under such conditions where the systemic vasodepressor effects of AT₁ receptor blockade do not occur, the powerful effects of AT₁ receptor blockade to increase renal blood flow and sodium excretion can be fully manifested.

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