Kidney Is an Important Target for the Antihypertensive Action of an Angiotensin II Receptor Antagonist in Spontaneously Hypertensive Rats

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Inhibitors of the renin-angiotensin system lower blood pressure of spontaneously hypertensive rats, although plasma renin is not elevated. To test the hypothesis that the actions of angiotensin II within the kidney may contribute to the high blood pressure in spontaneously hypertensive rats, we infused valsartan, a subtype 1 angiotensin II receptor antagonist, via the suprarenal artery into the right kidney of conscious, freely moving, unilaterally nephrectomized (left) spontaneously hypertensive rats (12 to 14 weeks old). Valsartan (0.3 mg/kg per day for 48 hours) lowered blood pressure (change in blood pressure, \(-7 \pm 3\), \(-19 \pm 4\), and \(-26 \pm 4\) mm Hg, n=11, at 12, 24, and 48 hours) after intrarenal administration but had no significant effect on blood pressure after intravenous administration (change in blood pressure, \(1 \pm 5\), \(-3 \pm 4\), and \(10 \pm 5\) mm Hg, n=1, at 12, 24, and 48 hours). Infusion of vehicle (0.9% saline) intrarenally had no significant effect on blood pressure (change in blood pressure, \(2 \pm 5\), \(-1 \pm 6\), and \(0 \pm 7\) mm Hg, n=11, at 12, 24, and 48 hours). The maximum fall in blood pressure reached after intrarenal administration of this dose of valsartan was similar to the maximum fall induced after intravenous administration of higher doses (change in blood pressure, \(-14 \pm 5\), \(-27 \pm 4\), and \(-32 \pm 5\) mm Hg, n=7, at 12, 24, and 48 hours after 3 mg/kg per day i.v.). Thus, endogenous angiotensin II acting within the kidney appears to play an important role in the maintenance of high blood pressure in spontaneously hypertensive rats. (Hypertension 1993;21:1056-1061)

KEY WORDS • kidney • blood pressure • rats, inbred SHR • angiotensin II • receptors, angiotensin • renin-angiotensin system

Several lines of experimental evidence indicate that the kidney plays a pivotal role in the etiology of hypertension in spontaneously hypertensive rats (SHR). Renal transplantation experiments have consistently shown that hypertension can be transferred with the kidneys from genetically hypertensive donors to normotensive recipients.\(^1\) Conversely, blood pressure (BP) is decreased or the development of hypertension prevented by transplantation of kidneys from genetically normotensive donors to hypertensive recipients.\(^1\) Renal function studies have shown that renal hemodynamic abnormalities may be involved in the initiation of hypertension in SHR. Young SHR (4 to 6 weeks) are moderately hypertensive and have a reduced glomerular filtration rate and renal blood flow and an increased renal vascular resistance compared with the normotensive Wistar-Kyoto control strain.\(^2\,3\) Moreover, an exaggerated salt and water retention has been detected in young SHR.\(^3\) As the hypertension develops, renal hemodynamic abnormalities become normalized (after 12 to 14 weeks of age). The low renal blood flow and glomerular filtration rate in young SHR may be a stimulus for BP to increase and return renal perfusion to normal.\(^3\)

SHR have a normal to low plasma renin activity\(^4\,5\) and have not generally been considered to be a renin-dependent model of hypertension.\(^6\) However, the antihypertensive efficacy of renin inhibitors,\(^7\) angiotensin converting enzyme (ACE) inhibitors,\(^7\,9\) and angiotensin II (Ang II) receptor antagonists\(^5\,10\) in SHR implicates an involvement of the renin-angiotensin system in the pathogenesis of hypertension in this animal model. Treatment of SHR with an ACE inhibitor normalizes glomerular filtration rate, renal blood flow, renal vascular resistance, and BP,\(^9\) suggesting a role for Ang II in both the renal hemodynamic abnormalities and development of hypertension. The observation that the antihypertensive response induced by renin inhibition, ACE inhibition, or Ang II receptor antagonists\(^5\,10\) in SHR implicates an involvement of the renin-angiotensin system in the pathogenesis of hypertension in this animal model. This conclusion is also supported by the observations that after withdrawal of ACE inhibitor treatment in SHR, the antihypertensive effect persists and is accompanied by a corresponding persistent inhibition of renal ACE activity\(^12\,13\) and a reduction in renal vascular resistance.\(^9\) In contrast, plasma ACE activity recovers rapidly. Although previous observations suggest that the actions of Ang II on the kidney are involved in the development and maintenance of high BP in SHR and that the kidney is an important site of action for...
Animals
Experiments were conducted with male SHR obtained from Iffa-Credo, Lyon, France. The rats received a standard rat chow (NAFAG, Gossau, Switzerland) and water ad libitum and were kept in a room with a 12-hour light/dark cycle (7 AM to 7 PM, light) at a temperature of 24°C. At the beginning of an experiment, rats were between 12 and 14 weeks of age and weighed 280 to 310 g. Under our laboratory conditions, hypertension has fully developed in these SHR after approximately 15 weeks of age.

Methods

Surgical Procedures
The surgical procedures used in these experiments for the implantation of arterial and venous catheters have previously been described in detail.5 With rats under halothane anesthesia, a catheter was inserted via a femoral artery into the aorta of all rats for the measurement of mean arterial pressure and for blood sampling. A catheter was also implanted into a femoral vein in rats that received intravenous infusions. Rats that received intrarenal infusions had a catheter inserted into the right suprarenal artery in addition to the arterial catheter. The tip of the renal catheter was situated at the junction of the suprarenal and renal arteries as described previously.14 In these rats, the contralateral kidney (left) was removed to enable the entire renal mass to be perfused with one catheter. All catheters were exteriorized at the neck. After recovery from anesthesia, the rats were kept in individual plastic cages. The catheters were protected by a spring and a plastic tubing. A continuous infusion of 0.9% saline containing 2 IU/mL heparin was delivered at a rate of 36 μL/hr through the arterial catheters immediately after surgery and for the duration of the experiment to ensure patency.

Measurement of Blood Pressure and Heart Rate
At least 2 days were allowed after surgery before BP was measured. Mean arterial BP and heart rates were measured continuously via the aortic catheter with an electronic transducer. The principle of differential pressure is based on the fact that the pressure drop over a catheter is proportional to the fluid flow rate when the fluid flow is laminar. The oscillograph was connected to a swivel. A continuous infusion of 0.9% saline was started at the time of surgery to provide the required volume of saline until all of the compound was dissolved. The pH of the solution was then backtitrated to 7.2 with 0.1N HCl. This solution was diluted to a final concentration of 10 mg/mL by the addition of saline. The stock solutions were diluted further with saline. Solutions were infused intravenously via the catheter in the femoral vein or intrarenally via the catheter in the suprarenal artery.

Experimental Protocols

Effect of valsartan after intravenous infusion. Initial experiments were designed to determine the effective dose range for lowering BP after intravenous administration of valsartan. The compound was infused in the following doses over 48 hours: 0.1 (n=3), 0.3 (n=7), 1.0 (n=6), 3.0 (n=7), and 10 (n=6) mg/kg per day. Control animals (n=7) received an infusion of the isotonic vehicle delivered at the same rate (60 μL/hr). BP and heart rate were measured for 24 hours before and for 48 hours after the infusion of drug or vehicle was started. The potent, selective, and competitive AT1 receptor antagonist valsartan, (S)-N-valeryl-N-[(2′-[1H-tetrazol-5-yl)biphenyl-4-yl]-methyl]-valine, was synthesized by CIBA GEIGY Ltd., Basel, Switzerland.17 Stock solutions of valsartan (10 mg/mL) were prepared in 0.9% saline as follows: drops of 0.1N NaOH were added to a suspension of valsartan in approximately half the required volume of saline until all of the compound was dissolved. The pH of the solution was then backtitrated to 7.2 with 0.1N HCl. This solution was diluted to a final concentration of 10 mg/mL by the addition of saline. The stock solutions were diluted further with saline. Solutions were infused intravenously via the catheter in the femoral vein or intrarenally via the catheter in the suprarenal artery.
after intravenous infusion (0.3 mg/kg per day, n=11) was chosen for intrarenal administration. The control group of rats (n=11) received vehicle intrarenally delivered at the same rate (60 μL/hr) for 48 hours. In these experiments, BP and heart rate were measured for 24 hours before and 48 hours after the infusions were started. To avoid effects of blood sampling on BP, we used separate groups of rats prepared in the same way (i.e., unilateral nephrectomy, intrarenal and intravenous catheters) to collect blood samples for the measurement of the plasma concentrations of valsartan, renin, hematocrit, plasma osmolality, and plasma electrolytes. These groups of rats received the following infusions for 48 hours: vehicle intravenously (n=10), valsartan 0.3 mg/kg per day intravenously (n=9), valsartan 0.3 mg/kg per day intravenously (n=9), or valsartan 3.0 mg/kg per day intravenously (n=6). Blood samples were collected at 2, 6, 18, 24, and 48 hours after the infusions were started. BP and heart rate were recorded continuously, and a challenge dose of Ang II was given after 48 hours of infusion to test the degree of peripheral Ang II receptor blockade.

Data and Statistical Analysis

Values given in the text and figures are mean±SEM. Statistical significance of treatment effects was evaluated using a trend analysis based on a covariance model including dose as a class variable and estimated baseline as a covariant. Differences between intravenous and intrarenal groups were assessed by testing for significant differences between linear terms for the trend analysis from the two groups. Evaluations were performed using the absolute data for the parameters measured. Effects were considered to be of statistical significance at a value of p<0.05 for a one-sided test.

Results

Effect of Intravenous Infusions of Valsartan on Blood Pressure

After intravenous infusion of valsartan, the threshold dose for BP lowering was approximately 1 mg/kg per day because the doses of 0.1 and 0.3 mg/kg per day had no significant antihypertensive effect (Figure 1). With doses of 1 mg/kg per day and above, the antihypertensive effect developed slowly over 24 hours to reach a maximum after 24 to 48 hours. The maximum lowering of BP in response to valsartan was obtained with doses between 3 and 10 mg/kg per day.

Effect of Intrarenal Infusion of Valsartan on Blood Pressure

Although BP was not significantly changed after intravenous administration of valsartan in a dose of 0.3 mg/kg per day, it was significantly lowered after intrarenal administration of this dose (Figure 2). The onset of the response was slow, and the maximum effect was reached after approximately 48 hours. The magnitude of the hypotensive response after 0.3 mg/kg per day given intrarenally was similar to that induced by 3 mg/kg per day given intravenously (Figure 2). BP was not significantly changed after intrarenal infusion of vehicle. Heart rate was not significantly changed during the intrarenal infusion of valsartan (317±4, 316±6, 318±10, 312±12, 297±8, and 282±8 beats per minute, n=11, at 0, 2, 6, 18, 24, and 48 hours, respectively).

Effect of Intrarenal or Intravenous Valsartan on the Pressor Response to Angiotensin II

Intravenous injection of Ang II produced an increase in BP of approximately 55±2 mm Hg (n=10) in the vehicle-treated rats. The pressor response to the intravenous injection of Ang II was blocked by 20% (p<0.05) after the intrarenal infusion of 0.3 mg/kg per day valsartan (increase in BP, 45±4 mm Hg, n=9) and by 38% (p<0.05) after the same dose of the AT1 blocker given intravenously (increase in BP, 35±5 mm Hg, n=9). After the intravenous infusion of valsartan at 3 mg/kg per day, the pressor response to Ang II was almost completely blocked (increase in BP, 4±2 mm Hg, n=6, p<0.05).
Effect of Intrarenal or Intravenous Valsartan on Plasma Valsartan Concentrations

Plasma valsartan concentrations were unmeasurable (<75 nM) in the rats that received either intravenous or intrarenal valsartan at 0.3 mg/kg per day. Plasma concentrations of valsartan were 515±43, 590±44, 500±26, 437±68, and 430±41 nM (n=6) at 2, 6, 18, 24, and 48 hours, respectively, after intravenous infusion of valsartan at 3 mg/kg per day.

Effect of Intrarenal or Intravenous Valsartan on Plasma Renin Concentrations

In control animals, plasma renin concentration was stable over the 48-hour experimental period (Figure 3). Plasma renin concentration increased significantly, after the valsartan dose of 3 mg/kg per day infused intravenously, to a maximum after approximately 6 hours (sevenfold). Plasma renin concentration also tended to increase within 2 hours after the dose of 0.3 mg/kg per day given either intravenously (twofold) or intrarenally (threefold), to reach a maximum after 6 hours, but the effect did not reach statistical significance. Plasma renin concentration began to decrease after 6 hours with the intravenous doses but remained slightly elevated in some animals after the intrarenal dose.

Effect of Intrarenal or Intravenous Valsartan on Plasma Hematocrit, Osmolality, and Electrolyte Concentrations

There was no significant effect of the treatments on hematocrit, plasma osmolality, or plasma sodium or potassium concentrations (data not shown).

Discussion

The purpose of this study was to determine whether at least part of the antihypertensive actions of an AT1 antagonist could be explained by inhibition of the actions of Ang II within the kidney. Intravenous infusion of the selective AT1 antagonist valsartan at doses between 1 and 10 mg/kg per day to 12 to 14-week-old SHR induced a slowly developing, dose-dependent reduction in BP over a 48-hour period. The maximum reduction in BP was reached at doses of 3 and 10 mg/kg per day. Lower doses (0.1 and 0.3 mg/kg per day) did not alter BP. A reduction in BP in response to systemic blockade of AT1 receptors in the SHR confirms previous observations5,6 and suggests that the renin-angiotensin system plays a major role in the elevated BP characteristic of this animal model. These results also show that the actions of Ang II that are responsible for the elevation of pressure in SHR are mediated via the AT1 receptor.

Ang II has many actions, including constriction of vascular smooth muscle, activation of the sympathetic nervous system, and potentiation of vascular smooth muscle growth, as well as effects on the kidney leading to a reduction of sodium and water excretion. Thus, the fall in BP in response to intravenous infusion of valsartan could be due to inhibition of the actions of Ang II at AT1 receptors on one or more of these systems. To determine the contribution of Ang II acting within the kidney to the antihypertensive actions of valsartan, we infused the AT1 antagonist intrarenally in a dose that was subthreshold for lowering BP when given intravenously (0.3 mg/kg per day). When infused intrarenally, this dose of valsartan induced a fall in BP of approximately 25 mm Hg. Indeed, the fall in BP produced by the intrarenal infusion of valsartan approached the maximum antihypertensive response that could be produced after intravenous administration of higher doses.

The AT1 antagonist valsartan has a long biological half-life in the rat, and therefore it could be expected that with time some compound might spill from the renal into the peripheral circulation. However, although antihypertensive when delivered to the kidney, the same dose did not lower BP when given intravenously. This observation supports a selective intrarenal effect. Further evidence in support of this is provided by the observations that plasma concentrations of valsartan were undetectable after both the intrarenal and intravenous dose of 0.3 mg/kg per day. There was some inhibition of the pressor response to Ang II after intravenous administration of valsartan (38%), even though this dose was not sufficient to influence baseline BP. After the same dose given intrarenally, the even smaller (20%) inhibition of the Ang II pressor response was probably due to blockade of the constrictor effects of Ang II on the renal vascular bed. It is one of the most sensitive vascular beds to the constrictor effects of Ang II, and the solitary kidney would have contributed approximately 10% to total peripheral resistance. The 10-fold higher dose of valsartan (3 mg/kg per day) delivered intravenously resulted in almost complete blockade of the Ang II pressor response and an antihypertensive response of magnitude similar to that of the intrarenally delivered dose. Thus, intrarenally administered valsartan appeared to have lowered BP by a primary interaction with intrarenal AT1 receptors.

Recently published data are in agreement with our findings.18 Ang II antagonists targeted to the kidney by administration of inactive prodrugs that are selectively activated intrarenally lower BP in SHR. The maximum antihypertensive response was similar to that observed in the present experiments. These findings implicate a role of Ang II acting on the kidney in the maintenance of high BP in SHR. However, the specific mechanisms involved are not clear. Although renin and Ang II concentrations are not elevated in the plasma in SHR, they may be within the kidney. Renal renin content has...
been reported to be elevated in very young SHR compared with Wistar-Kyoto rats in the first few weeks after birth but not at later time points.\textsuperscript{4,19} ACE activity and intrarenal Ang II concentrations are also reported to be higher in young SHR than in Wistar-Kyoto rats.\textsuperscript{20} Alternatively, changes at the receptor level, such as receptor density or affinity, or in postreceptor mechanisms might be involved. Ang II receptor density in both the brush border and glomerulus of young SHR are higher than in Wistar-Kyoto rats.\textsuperscript{21} An abnormality at the receptor or postreceptor level within the kidney is implicated by the observations that SHR are more sensitive to the slow-pressor effects of intrarenal infusion of a very low dose of Ang II.\textsuperscript{22}

The present study supports previous observations indicating that endogenous Ang II acting on the kidney is involved in the development and maintenance of high BP in SHR. Ang II has both direct and indirect actions at the kidney that promote the retention of sodium and water. It constricts the renal vasculature (probably a preferential effect on the efferent artery) and induces a lowering of both renal blood flow and glomerular filtration rate, which in turn may lead to retention of sodium and water. In addition, Ang II appears to have a direct effect in promoting sodium reabsorption in the proximal tubule. In previous studies, it has been shown that selective intrarenal blockade of the renin-angiotensin system in volume-depleted animals leads to increased renal blood flow and to sodium and volume excretion.\textsuperscript{23} Blockers of the renin-angiotensin system also increase renal blood flow and promote renal salt and volume loss in hypertensive animals.\textsuperscript{24,25} However, it has not been established whether sodium and volume retention is the primary mechanism whereby hypertension develops and whether the antihypertensive action of blockers of the renin-angiotensin system is due to reversal of these effects. Although in the present studies intrarenal infusion of valsartan lowered BP, hematocrit and the plasma concentrations of sodium and potassium were not affected after 48 hours. Thus, it remains to be determined whether intrarenal infusion of valsartan exerts its antihypertensive effect by promoting renal sodium excretion.

Another interesting aspect of the present studies is that the intrarenal infusion of the AT\textsubscript{1} receptor antagonist had little effect on plasma renin concentration. Other studies have shown that plasma concentrations of renin and Ang II are significantly increased after intravenous or oral administration of ACE inhibitors or AT\textsubscript{1} Ang II receptor antagonists.\textsuperscript{26} Indeed, in the present study, a significant increase in plasma renin concentration was observed after an intravenous dose that lowered BP to a similar extent as the intrarenal dose. Renin release from the kidney is under the control of several factors, including changes in BP and the negative feedback of Ang II on the juxtaglomerular cells. Because in the present study the fall in BP was similar with the intrarenal and a 10-fold higher intravenous dose, BP cannot explain the difference in effect on plasma renin concentration. Intrarenal infusion of valsartan may have affected other mechanisms of renin release that were not disrupted during intravenous infusion. The increase in plasma renin after the intravenous dose was only transient. The peak reached after 6 hours may have been due to release of all of the stored renin. The levels may have decreased thereafter because all of the stored renin was depleted and only newly synthesized renin was being released. It may require a longer time for renin synthesis to be increased.

In summary, these results demonstrate that the intrarenal actions of Ang II contribute to the elevated BP in SHR and that the kidney is an important target for the antihypertensive action of AT\textsubscript{1} Ang II receptor antagonists. These findings may also explain, at least in part, why blockers of the renin-angiotensin system lower BP in SHR although plasma Ang II concentrations are not elevated.

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\textbf{References}

Wood et al Intrarenal Angiotensin II Antagonism


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