Renal Nitric Oxide and Angiotensin II Interaction in Renovascular Hypertension

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In two-kidney, one clip (2K1C) renovascular hypertension, blood flow is reduced to the clipped but not to the nonclipped kidney, despite elevated angiotensin II. To determine possible interactions between endothelium-derived nitric oxide and angiotensin, we studied bilateral renal blood flow using radioactive microspheres in anesthetized 2K1C hypertensive rats 4 weeks after clipping. We studied the response to nitric oxide synthesis inhibition with 10 mg/kg body wt N\textsuperscript{G}-nitro-L-arginine-methyl ester (L-NAME) in hypertensive rats untreated (n=5) or treated (n=5) with 10 mg/kg body wt of the angiotensin II antagonist losartan. 2K1C rats had a blood pressure of 159±9 mm Hg, and renal blood flow to the clipped kidney was reduced 87% compared with the nonclipped kidney. L-NAME increased blood pressure 36±5 mm Hg and decreased renal blood flow in the nonclipped kidney 61% (4.9±0.5 to 1.9±0.4 mL/min per gram kidney weight, P<.001). Renal vascular resistance increased 200% (33.4±2.2 to 100.7±15.0 resistance units [RU], P<.001). Renal blood flow and resistance in the clipped kidney were unchanged by L-NAME. Treatment of 2K1C rats with losartan reduced blood pressure (154±8 to 116±11 mm Hg, P<.01), did not change blood flow in the nonclipped, but normalized it in the clipped kidney (4.8±0.8 mL/min per gram kidney weight). With losartan, L-NAME increased pressure by 38±10 mm Hg, decreased renal blood flow 10% (5.9±0.5 to 5.3±0.3 mL/min per gram kidney weight, P<.05), and increased renal resistance 49% (19.7±1.2 to 29.4±2.2 RU, P<.001) in the nonclipped kidney. Neither blood flow nor resistance was changed in the clipped kidney. Our results suggest that nitric oxide maintains renal perfusion of the nonclipped but not the clipped kidney in 2K1C rats. Nitric oxide counteracts elevated angiotensin to regulate perfusion in the nonclipped kidney, but angiotensin vasoconstriction predominates in the clipped kidney. Thus, nitric oxide vasodilation is a regulatory response to maintain contralateral renal perfusion despite elevated angiotensin after renal artery stenosis in 2K1C hypertension. (Hypertension 1993;22:237-242)

KEY WORDS • nitric oxide • endothelium • angiotensin II • renal circulation • losartan • vascular resistance • hypertension, renovascular

It has been suggested that various forms of hypertension are characterized by a dysfunctional endothelium that contributes to the rise in blood pressure.\textsuperscript{1-6} In models including two-kidney, one clip (2K1C) Goldblatt, aortic coarctation, Dahl salt-sensitive, and deoxycorticosterone acetate-salt hypertension, as well as spontaneously hypertensive rats, endothelium-dependent vasodilation is impaired\textsuperscript{1-3} but may be restored by reverting the hypertension.\textsuperscript{1} Similar abnormalities have been reported in humans with essential hypertension. These reports suggest that endothelial dysfunction associated with hypertension may be due to insufficient endothelium-derived nitric oxide (EDNO). In normotensive subjects, EDNO has been found to be an important mediator of renal blood flow (RBF).\textsuperscript{7,8} Inhibition of EDNO synthesis using either N\textsuperscript{G}-nitro-L-arginine-methyl ester (L-NAME) or N\textsuperscript{G}-monomethyl L-arginine results in decreased RBF\textsuperscript{7,8} and increased renal vascular resistance (RVR). This increase in RVR is suggested to be the result of removing intrinsic EDNO-mediated renal vasodilation, allowing endogenous vasoconstrictors, such as angiotensin II (Ang II), to predominate. The renal response occurs simultaneously with a rise in systemic pressure, suggesting that EDNO is important in maintaining both systemic and renal resistances.

Using anesthetized rats, we have previously shown that blocking the renin-angiotensin system with either converting enzyme inhibition or an Ang II receptor antagonist eliminated the decrease in RBF and attenuated the increase in RVR observed after treatment with L-NAME.\textsuperscript{7,9,10} However, Ang II blockade did not impair the systemic response to EDNO synthesis inhibition. We concluded that within the renal vasculature there is a unique interaction between the vasodilator influence of EDNO and the vasoconstrictor influence of Ang II. Therefore, it seemed likely that development of an Ang II-dependent form of hypertension could be attributed to an imbalance between Ang II and EDNO. Since Goldblatt et al\textsuperscript{11} demonstrated the development of hypertension after renal stenosis, the pathogenesis of renovascular hypertension has been studied extensively.\textsuperscript{12} Clipping the renal artery results in an immediate fall in RBF and glomerular filtration rate (GFR) in the clipped kidney, whereas plasma renin activity (PRA) and blood pressure (BP) increase.\textsuperscript{12,13}
Within 4 weeks, PRA increases fivefold to 10-fold, and the rats become hypertensive. During this early phase of developing hypertension, the balance between BP and plasma volume is altered, presumably because of changes in renal hemodynamics and increased renal nerve activity. In addition, various functional abnormalities have been reported in the contralateral, nonclipped kidney in 2K1C hypertension. Ploth et al reported that the nonclipped kidney cannot autoregulate RBF and that pressure natriuresis is blunted. Also, contralateral RVR is increased as the result of an increase in both afferent and efferent arteriolar vasoconstriction. However, by 4 weeks RBF and GFR in the nonclipped kidney (per gram of kidney weight) are similar to that seen in normotensive controls despite the increased BP, RVR, and elevated circulating Ang II. How the nonclipped kidney maintains normal function is not understood. Because we have observed that the balance between Ang II and EDNO is important in normal renal hemodynamics, it seemed likely that it could be a significant factor in this Ang II-dependent model of hypertension. After clipping, the increased renal perfusion should cause shear stress to rise in the nonclipped kidney, a primary stimulus for EDNO synthesis. Therefore, we hypothesized that in 2K1C renovascular hypertension, RBF to the nonclipped kidney is maintained at essentially normal levels because of increased EDNO production that counterbalances the constrictor influence of elevated local and circulating Ang II.

Methods

2K1C hypertension was induced as described previously. Briefly, male Sprague-Dawley rats weighing 180 to 200 g were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Ill.). Under antiseptic conditions, the left renal artery was exposed through a retroperitoneal flank incision and carefully dissected free of the renal vein. A silver clip (wood Co, Milwaukee, Wis) and placed on a heating pad before the experiment.

Four weeks after clipping, the rats were fasted overnight and allowed free access to water. The rats were anesthetized by an intraperitoneal injection of 125 mg/kg body wt thiobutabarbital (Inactin, Andrew Lockwood Co, Milwaukee, Wis) and placed on a heating pad to maintain constant body temperature. A PE10 catheter (Fisher Scientific, Chicago, Ill.) was inserted into the right common carotid artery and passed into the left ventricle. The position of the catheter tip in the left ventricle was adjusted until the left ventricular pulse pressure could be read without artifacts. The right femoral vein and artery were catheterized with PE50 tubing (Fisher Scientific). The venous catheter was used for constant infusion of saline (40 µL/min), infusion of drugs, and blood replacement, and the arterial catheter was used to monitor BP and for reference blood sampling. BP was recorded with a Statham pressure transducer (Vigo-Spectramed, Oxnard, Calif) connected to a chart recorder (Gould, Inc, Valley View, Ohio). After surgery, the rats were allowed a 60-minute stabilization period during which BP was monitored.

The effect of EDNO synthesis inhibition on RBF, RVR, cardiac output (CO), and total peripheral resistance (TPR) was measured with radioactive microspheres (Du Pont-New England Nuclear, Boston, Mass) with a diameter of 15±1.5 µm and labeled with either 14Ce or 85Sr. (Using two isotopes allows us to carry out paired measurements before and after treatment.) Because of the anaphylactic response of rats to the commercial dextran vehicle, resulting in severe hypotension, we modified the protocol by suspending microspheres in 3.5 M glucose using 0.01% Tween 80 as an antiaggregant. This concentration of glucose and Tween 80 had no effect on systemic pressure. Microspheres at a concentration of 400 000/mL were ultrasonically agitated for approximately 15 minutes. A volume of 0.2 mL of the suspension, corresponding to approximately 80 000 microspheres, was then drawn up into a syringe. The syringe was counted to obtain the preinjection dose and connected to the left ventricular catheter. The microspheres, together with 0.2 mL saline, were then infused into the left ventricle over 20 seconds while a reference arterial blood sample was simultaneously withdrawn at a rate of 0.48 mL/min for 75 seconds. The withdrawn blood was replaced with heparinized blood obtained from a donor rat nephrectomized 16 to 24 hours earlier. The injection syringe was again counted after microsphere injection to obtain the postinjection dose, and the injection dose was obtained by subtracting the preinjection from the postinjection dose. For the determination of the effect of EDNO synthesis inhibition on renal hemodynamics, microspheres were injected 15 minutes after EDNO synthesis inhibition with L-NAME. We have found that systemic and renal inhibition of EDNO synthesis with this dose is complete within 10 minutes after administration. Five minutes after the microspheres were injected, the animals were killed with 150 mg/kg IV sodium pentobarbital; the kidneys were removed, weighed, and counted in a Packard gamma counter using a dual window setting of 10 to 250 and 400 to 700 meV at a sample level of 0.5 cm.

RBF (milliliters per minute per gram kidney weight), RVR (millimeters of mercury per milliliter per minute per gram kidney weight) (referred to as resistance units or RU), CO (milliliters per minute per 100 g body weight), and TPR (millimeters of mercury per milliliter per minute per 100 g body weight) (RU) were determined as follows: (1) RBF=counts per minute (cpm) organ x pump speed/cpm blood x gram kidney weight, (2) RVR=mean BP/RBF, (3) CO=cpm injected x pump speed/cpm blood x body weight/100 g, (4) TPR=mean BP/CO. All results are expressed as mean±SEM for each rat group. Changes induced by drug treatment were analyzed with Student’s paired t test. Nonpaired parameters were compared using Student’s t test. A value of P<.05 was considered significant. The protocol of this study was approved by our institutional animal care review committee.

A dose of 10 mg/kg body wt of L-NAME (Sigma Chemical Co, St Louis, Mo) was used to inhibit EDNO synthesis. We have previously documented that this dose induces sustained inhibition of EDNO synthesis in...
the systemic and renal vasculatures. The experiments were divided into two groups as described below.

**Effect of Endothelium-Derived Nitric Oxide Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics**

Four weeks after clipping, 2K1C hypertensive rats were prepared as described above. After surgery, the rats were allowed a 60-minute recovery period during which BP was recorded. After this period, or when BP stabilized, baseline values (n=5) were obtained by injecting one set of microspheres as detailed above.

For the determination of the influence of EDNO synthesis inhibition on systemic and renal hemodynamics, rats were treated with L-NAME. Fifteen minutes later, when BP was stable at its new level, microspheres were administered. After these procedures, the animals were killed and the kidneys excised, weighed, and counted.

**Effect of Endothelium-Derived Nitric Oxide Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics After Angiotensin II Blockade**

Four weeks after clipping, 2K1C hypertensive rats were prepared as described above. After surgery, the rats were allowed a 30-minute recovery period during which BP was recorded. After this period, or when BP stabilized, the rats received 10 mg/kg body wt of the nonagonistic Ang II receptor antagonist losartan (DuP 753, Pu Pont Corp, Wilmington, Del), and BP was again recorded for 30 minutes. After this second 30-minute period, or when BP stabilized, baseline values (n=5) were obtained by injecting one set of microspheres as detailed above.

For the determination of the effect of EDNO synthesis inhibition on systemic and renal hemodynamics after losartan administration, rats were treated with L-NAME. Fifteen minutes later, when BP was stable at its new level, microspheres were administered. After these procedures, the animals were killed and the kidneys excised, weighed, and counted.

**Results**

**Effect of Endothelium-Derived Nitric Oxide Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics**

Basal BP of the 2K1C hypertensive rats was 159±9 mm Hg, CO was 25.1±3.4 mL/min per 100 g body weight, and TPR was 6.6±0.6 RU. The changes in BP, CO, and TPR are shown in Fig 1 (open circles). L-NAME significantly increased BP by 35±6 mm Hg (P<.001), decreased CO by 39% (to 15.2±0.9 mL/min per 100 g body weight, P<.025), and increased TPR by 59% (to 10.5±0.6 RU, P<.005).

Basal RBF in the nonclipped kidney of 2K1C hypertensive rats was 4.92±0.46 mL/min per gram kidney weight compared with only 0.63±0.31 mL/min per gram kidney weight in the clipped kidney. The RVR of the nonclipped kidney was 33.4±2.2 RU, compared with 884.6±333.1 RU in the clipped kidney. The changes in RBF and RVR in the nonclipped and clipped kidneys in response to L-NAME are shown in Figs 2 and 3. In the nonclipped kidney, L-NAME decreased RBF by 61% (P<.005) and increased RVR by 200% (P<.005), whereas in the clipped kidney, L-NAME had no significant effect on either RBF or RVR.

**Effect of Endothelium-Derived Nitric Oxide Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics in Rats Pretreated With Losartan**

Basal BP of the 2K1C hypertensive rats was 154±8 mm Hg. Treatment with losartan decreased BP by 38±4 mm Hg, to 116±11 mm Hg. In rats pretreated with losartan, CO was 31.7±3.0 mL/min per 100 g body weight, and TPR was 3.7±0.3 RU. The changes in BP, CO, and TPR to L-NAME are shown in Fig 1. L-NAME significantly increased BP by 38±8 mm Hg (P<.01), decreased CO by 28% (to 22.9±2.6 mL/min per 100 g body weight, P<.001), and increased TPR by 91% (to 7.1±0.8 RU, P<.005). All of the changes induced by L-NAME were similar and parallel to those seen above.

After treatment with losartan, basal RBF in the nonclipped kidney of 2K1C hypertensive rats was 5.91±0.46 mL/min per gram kidney weight, which was not different from RBF in the nonclipped kidney of the
FIG 2. Plots show changes in renal blood flow and renal vascular resistance in nonclipped kidney of two-kidney, one clip hypertensive rats (n=5) in response to 10 mg/kg body wt N^6-nitro-L-arginine-methyl ester (L-NAME), an inhibitor of endothelium-derived nitric oxide synthesis, in the presence and absence of 10 mg/kg body wt losartan. *Significant change induced by L-NAME (P<.05). gkw, Gram kidney weight.

non-losartan-treated group. However, RBF in the clipped kidney of losartan-treated rats (4.85±0.76 mL/min per gram kidney weight) was sevenfold higher than that in the non-losartan–treated group and not different from the nonclipped kidney. RVR in the nonclipped kidney was reduced by 40% by losartan, to 19.7±1.1 RU (P<.001), whereas in the clipped kidney, losartan decreased RVR by an order of magnitude, to 25.6±2.9 RU.

In losartan-treated rats, the changes induced by L-NAME in RBF and RVR in the nonclipped and clipped kidneys are shown in Figs 2 and 3. In the nonclipped kidney, L-NAME decreased RBF by only 10% (P<.05) and increased RVR by only 49% (P<.005). Each of these changes was only approximately 25% of that seen in rats not treated with losartan (above, P<.001). In the clipped kidney, neither RBF nor RVR changed after L-NAME.

**Discussion**

In 2K1C renovascular hypertension, renal artery stenosis produced with a clip results in a fall in renal perfusion to the clipped kidney, a rise in PRA and circulating Ang II, and a steady increase in BP. These events are accompanied by a compensatory response of the contralateral nonclipped kidney, so that its perfusion is normalized despite the increased perfusion pressure and elevated Ang II. Elevated Ang II should increase RVR, and a rise in BP should increase renal perfusion, resulting in greater vascular shear stress, which is a stimulus for EDNO synthesis. We have previously found that renal perfusion in normotensive rats is controlled by a balance between the effects of Ang II and EDNO. Thus, we hypothesized that RBF in the nonclipped kidney of 2KIC rats could also be maintained because of increased EDNO production, counterbalancing the constrictor influence of the elevated Ang II. Our results support this hypothesis. We found that EDNO synthesis inhibition with L-NAME resulted in significantly exaggerated changes in RBF and RVR in the nonclipped kidney. We found that L-NAME resulted in twice the decrease in RBF as well as an increase in RVR that was 2.5 times greater compared with the changes we have reported in kidneys of normotensive rats. This suggests that the role of EDNO in maintaining renal hemodynamics was greatly amplified compared with that seen in kidneys of normotensive rats, including those with chronically elevated Ang II. We do not find evidence that a dysfunctional endothelium is a permissive characteristic of
vasoconstriction. Ploth et al. reported that the contra-
culcular hypertension, the degree of stenosis may vary
stenotic kidney in our model. In humans with renovas-
tion, suggesting that EDNO synthesis balances the
increase in RVR seen with EDNO synthesis inhibi-
tion, suggesting that EDNO synthesis balances the
renal vasoconstrictor effect of Ang II and thereby main-
tains RBF. Based on what we have seen in normotensive rats, we hypothesized that in 2K1C hypertension with elevated Ang II, Ang II blockade should result in decreased BP and significant attenuation of the renal response to L-NAME. We found that this was indeed the case; as BP was normalized, the decrease in RBF in the nonclipped kidney after L-NAME was less than 20% of that observed in 2K1C rats not given losartan. Thus, we believe the reason the nonclipped kidney of 2K1C rats (at 4 weeks) can have normal renal hemodynamics despite greatly elevated Ang II and increased renal perfusion pressure is due to an exaggerated compensatory response of EDNO synthesis within the renal vasculature. It is not clear what effect EDNO would have on renal function in the nonclipped kidney; however, a number of investigators have described functional abnormalities in the nonclipped kidney in 2K1C hypertension. Four weeks after clipping, GFR and RBF (per gram kidney weight) in the nonclipped kidney are similar to that seen in normotensive controls, and the rats achieve a new state of sodium balance. Increased RVR is the result of elevated afferent and efferent arteriolar vasoconstriction. Ploth et al. reported that the contralateral kidney cannot autoregulate RBF and pressure natriuresis is blunted. However, the role of nitric oxide in the adaptation of filtration and excretion in the nonclipped kidney remains to be described.

In the clipped or stenotic kidney, reduced renal perfusion is the initiating stimulus for the development of hypertension. In our rats, we found that RBF was reduced 80% to 90% 4 weeks after clipping even though the kidneys were still perfused. The clipped kidney has high local Ang II levels and is the origin of elevated circulating Ang II; however, unlike its nonclipped counterpart, its reduced perfusion should diminish shear stress and pressure stimuli for EDNO. Thus, we hypothesized that the clipped kidney should produce little EDNO, so that its circulation is subjected primarily to the vasoconstrictor influence of elevated local and circulating Ang II. We found that EDNO synthesis inhibition had no significant effect on either RBF or RVR in the clipped kidney, in contrast to the exaggerated responses seen in the nonclipped kidney. These data suggest that EDNO has little effect within the stenotic kidney in our model. In humans with renovascular hypertension, the degree of stenosis may vary more than in our uniform experimental model. Thus, unlike the apparent absence of EDNO in our clipped kidneys, there would likely be a spectrum of diminished influence of EDNO, partially preserving perfusion of the compromised kidney.

If renal perfusion in the stenotic kidney is largely mediated by Ang II secondary to the clip, then unlike the contralateral kidney we expected that losartan would improve RBF. What we found was that even though BP dropped by 38 mm Hg, RBF increased more than sevenfold to essentially normal levels (per gram kidney weight), whereas RVR decreased to a fraction of its original value. This suggests that within 4 weeks after clipping, Ang II is the dominant factor in controlling perfusion of the clipped kidney, whereas the clip itself has become a secondary influence. Interestingly, EDNO synthesis inhibition did not further change the response of the clipped kidney after losartan treatment, similar to what we have observed in normotensive rats after Ang II blockade.

During the early phase of 2K1C hypertension, while BP is increasing (up to 4 weeks after clipping), the pathogenesis of the disease has been attributed to a combination of elevated Ang II, altered plasma volume, changes in renal function, and increased renal nerve activity. We found that in 2K1C hypertension at 4 weeks, EDNO synthesis inhibition increased BP by 36 mm Hg, whereas in normotensive rats under the same conditions, a similar dose of L-NAME increased BP by only 21 mm Hg. Interestingly, this exaggerated pressor response in the hypertensive rats was not altered by losartan, despite the decrease in systemic pressure or the dramatic effect on renal hemodynamics. Thus, blocking Ang II seems to dissociate the renal and systemic responses to L-NAME, suggesting that the Ang II–EDNO interaction is more important in the renal circulation than as a mediator of TPR. This dissociation of renal and systemic responses was also seen in normotensive rats. The endothelium is a rich source of both vasodilator and vasoconstrictor factors. EDNO is an important mediator of vascular tone and can be stimulated by a number of chemical and physical factors. We have previously reported that the systemic pressor response to L-NAME is exaggerated in various models of hypertension, regardless of the intrinsic involvement of the renin-angiotensin system. Thus, it is likely that the exaggerated systemic pressor response to L-NAME may be a function of elevated systemic resistance in hypertension but not necessarily because of Ang II. The inability of losartan to modify the systemic response to L-NAME suggests that interactions between nitric oxide and other intrinsic vasoconstrictors besides Ang II contribute to the systemic resistance. The exaggerated pressor response to L-NAME also implies that the endothelium is probably not dysfunctional in 2K1C hypertension (4 weeks after clipping), as previously suggested by in vitro studies.

In conclusion, our data suggest that in the early phase (4 weeks) of 2K1C renovascular hypertension, EDNO synthesis increases in the nonclipped kidney, presumably related to increased shear stress, which counteracts the constrictor influence of elevated circulating Ang II. Thus, blocking Ang II eliminates the exaggerated responses to EDNO synthesis inhibition in the nonclipped kidney. Conversely, in the clipped kidney, the vasoconstrictor effect of Ang II predominates, and EDNO does not appear to contribute to its perfusion. Blocking Ang II remarkably normalizes hemodynamics but still does
not elicit any response to L-NAME. The exaggerated systemic pressor response to EDNO synthesis inhibition in 2K1C hypertension remains intact with or without Ang II blockade, suggesting dissociation of the factors that mediate renal and systemic resistances. These observations suggest that there is a unique and important interaction between Ang II and EDNO in the regulation of renal hemodynamics. This interaction is lost in the clipped kidney but represents a fundamental factor preserving renal function in the nonclipped kidney.

Acknowledgments

This work was supported by grant HL-46683-A02 from the National Institutes of Health, Bethesda, Md. We thank Du Pont Corp for generously supplying us with the losartan. Losartan is now produced by Du Pont Pharma/Merck Sharp & Dohme.

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doi: 10.1161/01.HYP.22.2.237

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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