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{\textdagger}-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<.005). 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.1-6 In models including two-kidney, one clip (2K1C) Goldblatt, aortic coarctation, Dahl salt-sensitive, and deoxycorticosterone acetate-salt hypertension, as well as in spontaneously hypertensive rats, endothelium-dependent vasodilation is impaired but may be restored by reverting the hypertension.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).7,8 Inhibition of EDNO synthesis using either N\textsuperscript{\textdagger}-nitro-L-arginine-methyl ester (L-NAME) or N\textsuperscript{\textdagger}-monomethyl L-arginine results in decreased RBF7,8 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.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 demonstrated the development of hypertension after renal stenosis, the pathogenesis of renovascular hypertension has been studied extensively.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.
Within 4 weeks, PRA increases fivefold to 10-fold, and the rats become hypertensive.\(^\text{12}\) 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.\(^\text{14,15}\) In addition, various functional abnormalities have been reported in the contralateral, nonclipped kidney in 2K1C hypertension. Ploth et al\(^\text{16}\) 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.\(^\text{12}\) However, by 4 weeks RBF and GFR in the nonclipped kidney (per gram of kidney weight) are similar to that seen in normotensive controls\(^\text{12,17}\) 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.\(^\text{18}\) 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 the elevated circulating Ang II. In contrast, the stenotic or clipped kidney exhibits reduced perfusion, diminished shear stress, and consequently diminished stimulus for EDNO. This leaves the clipped kidney subjected primarily to the constrictor influence of elevated local and circulating Ang II.

**Methods**

2K1C hypertension was induced as described previously.\(^\text{11}\) 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 with an internal diameter of 0.23 mm was placed around the left renal artery, causing partial occlusion. The wound was closed and the rat allowed to recover for 4 weeks 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.

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 30-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 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.

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±0.48 mL/min per gram kidney weight 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 Nω-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.18 We have previously found that renal perfusion in normotensive rats is controlled by a balance between the effects of Ang II and EDNO.9,10 Thus, we hypothesized that RBF in the nonclipped kidney of 2K1C 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.9,10 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.10 We do not find evidence that a dysfunctional endothelium is a permissive characteristic of
vasoconstrictor. We reported that the contralateral hypertension, the degree of stenosis may vary
stenotic kidney in our model. In humans with renovascular
data suggest that EDNO has little effect within 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 de-
creased 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 compensa-
tory response of EDNO synthesis within the renal vas-
culature. It is not clear what effect EDNO would have on
renal function in the nonclipped kidney; however, a
number of investigators have described functional abnor-
malities 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 contra-
lateral kidney cannot autoregulate RBF and pressure
natriuresis is blunted. However, the role of nitric oxide in
the adaptation of filtration and excretion in the non-
clipped 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 coun-
terpart, 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 primar-
ily 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 exaggerat-
ed responses seen in the nonclipped kidney. These
data suggest that EDNO has little effect within the
stenotic kidney in our model. In humans with renovas-
cular 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
cipping, 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
to 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 renin 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 al-
tered 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 intrin-
sic involvement of the renin-angiotensin system. Thus,
it is likely that the exaggerated systemic pressor re-
sponse 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 inter-
actions between nitric oxide and other intrinsic vasocon-
strictors 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
cipping), as previously suggested by in vitro studies. 1
In conclusion, our data suggest that in the early phase
(4 weeks) of 2K1C renovascular hypertension, EDNO
synthesis increases in the nonclipped kidney, presum-
ably related to increased shear stress, which counteracts
the constrictor influence of elevated circulating Ang II.
Thus, blocking Ang II eliminates the exaggerated re-
sponses to EDNO synthesis inhibition in the nonclipped
kidney. Conversely, in the clipped kidney, the vasocon-
strictor 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.

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