Influence of Nitric Oxide in the Chronic Phase of Two-Kidney, One Clip Renovascular Hypertension

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Abstract—Chronic two-kidney, one clip (2K1C) renovascular hypertension is characterized by a largely angiotensin-independent elevated blood pressure (BP). We hypothesized that the long-term effect of hypertension would compromise endothelium-derived nitric oxide (NO) and diminish its influence in controlling renal perfusion. We determined the influence of endothelium-derived NO on renal hemodynamics and the angiotensin–NO interaction regulation of renal perfusion in rats with chronic 2K1C hypertension. Renal blood flow (RBF) was measured by radioactive microspheres in rats with either early-phase (4 weeks after clipping, n = 7) or chronic-phase (13 to 16 weeks after clipping, n = 7) 2K1C hypertension. The systemic and renal response to NO synthesis inhibition was determined with 10 mg/kg body wt Nω-nitro-L-arginine methyl ester (L-NAME). In rats with early-phase 2K1C hypertension, BP was 149 ± 3 mm Hg, which increased by 42 ± 3 mm Hg with L-NAME (P < .001). L-NAME decreased RBF by 20% (P < .02) and 17% (P < .005) and increased renal vascular resistance (RVR) by 58% (P < .005) and 62% (P < .02) in the nonclipped and clipped kidneys, respectively. In rats with chronic 2K1C hypertension, BP was 166 ± 3 mm Hg, and L-NAME increased this by 35 ± 6 mm Hg (P < .001). In the nonclipped and clipped kidneys of chronic 2K1C hypertensive rats, L-NAME decreased RBF by 20% (P < .01) and 17% (P < .01) and increased RVR by 51% (P < .005) and 60% (P < .02), respectively. There were no differences in L-NAME–induced changes between early- and chronic-phase 2K1C hypertensive rats. Next, we treated seven chronic-phase 2K1C hypertensive rats with 10 mg/kg body wt losartan, which reduced BP by only 7.7% (P < .005). After losartan, L-NAME increased BP by 41 ± 3 mm Hg (P < .001), decreased RBF to the nonclipped kidney by 44% (P < .05), and increased RVR by 110% (P < .005); the decrease in RBF was significantly greater compared with untreated chronic-phase controls (P < .05). In the clipped kidney, L-NAME decreased RBF by 26% (P < .05) and increased RVR by 76% (P < .05). Thus, angiotensin blockade did not attenuate the systemic or renal vasoconstriction to L-NAME. Our results suggest that in both early and chronic phases of 2K1C hypertension, NO contributes significant dilator tone to buffer the hypertension and maintains perfusion of both kidneys by counterbalancing angiotensin-independent vasoconstriction. (Hypertension. 1998;31:649–656.)

Key Words: renovascular hypertension ■ nitric oxide ■ angiotensin ■ blood pressure ■ renal perfusion

The potent endothelium-derived vasodilator NO has been found to be an important regulator of RBF.1,2 Inhibition of NO synthesis with competitive substrate antagonists such as L-NAME or Nω-monomethyl-L-arginine results in decreased RBF and increased RVR.1,2 It has been suggested that this increase in RVR is the result of eliminating intrinsic NO-mediated renal vasodilation, allowing endogenous vasoconstrictors such as Ang II to predominate.3 The renal vasoconstrictor response occurs simultaneously with a rise in systemic pressure, suggesting that (endothelium-derived) NO is important in maintaining both systemic pressure and RVR. Using anesthetized rats, we have previously shown that blocking the renin-angiotensin system with either a converting enzyme inhibitor or an Ang II receptor antagonist blunts the decrease in RBF and concomitant increase in RVR caused by treatment with L-NAME.1,2,4 However, elimination of the constrictor effect of Ang II did not impair the systemic pressor response to NO synthesis inhibition. From these observations, we concluded that within the renal vasculature there is a uniquely sensitive interaction between the vasodilator influence of NO and the vasoconstrictor influence of Ang II.3,4

It has been proposed that various forms of hypertension are characterized by a dysfunctional endothelium. It is suggested that a deficient production of endothelium-derived NO results in diminished vasodilator tone, allowing vascular resistance to increase, and this contributes to the elevated BP.5–10 In vitro observations of isolated vessels in a number of experimental models, including 2K1C Goldblatt renovascular hypertensive rats, rats with aortic coarctation, Dahl salt-sensitive rats, and rats with deoxycorticosterone acetate–salt hypertension, as well as spontaneously hypertensive rats, have demonstrated that endothelium-dependent vasodilation is impaired4,7 but may be normalized by antihypertensive therapy.6 Similar abnormalities in endothelial integrity have been reported in vivo both in spontaneously hypertensive rats7 and in humans with essential hypertension.11
hypertension. These findings suggest that the endothelial dysfunction contributes to the hypertension because of a reduced production of NO. However, in vivo studies have demonstrated that in the early phase of 2K1C hypertension, when increased BP is highly dependent on elevated Ang II (3 to 5 weeks after clipping), the dilator effect of NO does not appear to be reduced and may actually be increased in animals with unilateral renal artery stenosis. In this model of hypertension, inhibition of NO synthesis resulted in an exaggerated increase in systemic BP and a decrease in RBF in the nonclipped contralateral kidney. Such changes suggest that an increase in NO could be a compensatory response to the elevated TPR caused by elevated circulating Ang II. However, persistent long-term (chronic) renovascular hypertension is characterized by high BP and a diminished depressor response to acute Ang II blockade. It has been suggested that sustained hypertension results in endothelial damage or dysfunction and that the resulting decrease in NO exacerbates the underlying hypertension. Our studies were designed to determine in chronic 2K1C hypertension whether NO still exerts a significant influence on systemic and renal hemodynamics 13 to 16 weeks after clipping. We hypothesized that sustained or chronic 2K1C renovascular hypertension would be characterized by a diminished vasodilator influence of NO and that this would result in a further decrease in renal perfusion and function. Additionally, if chronic 2K1C hypertension has a diminished response to Ang II, we expect that the depressor response to acute Ang II blockade would also be blunted and that, furthermore, the regulation of renal perfusion would no longer be controlled by the interaction between Ang II and NO.

**Methods**

2K1C renovascular hypertension was induced as described previously. Briefly, male Sprague-Dawley rats weighing 175 to 200 g were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories). 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, resulting in partial occlusion of renal perfusion. The wound was closed, and the rat was allowed to recover for either 4 weeks (early-phase 2K1C renovascular hypertension) or 13 to 16 weeks (chronic-phase 2K1C renovascular hypertension).

Both early- and chronic-phase 2K1C renovascular hypertensive rats were fasted overnight but allowed free access to water. They were then anesthetized by an intraperitoneal injection of 125 mg/kg body wt thiobutabarbital (Inactin, Research Biochemical Inc) and placed on a heating pad to maintain constant body temperature. A PE-10 catheter (Fisher Scientific) was inserted into the right common carotid artery and passed into the left ventricle. The position of the catheter tip was adjusted until the left ventricular pulse pressure could be read without artifacts. The right femoral vein and artery were catheterized with PE-50 tubing (Fisher Scientific). The venous catheter was used for constant infusion of saline (40 μL/min), infusion of drugs, and blood replacement. The arterial catheter was used to monitor systemic BP and to sample blood. BP was recorded with a Statham pressure transducer (Vigo-Spectramed) connected to a chart recorder (Gould Inc). After surgery, the rats were allowed a 60-minute stabilization period during which BP was monitored.

The influence of NO on systemic BP and renal perfusion was evaluated with an in vivo bioassay consisting of monitoring the hemodynamic responses to NO synthesis inhibition with 10 mg/kg L-NAME. The effect of NO synthesis inhibition on RBF, RVR, CO, and TPR was measured by evaluating the distribution of radioactive microspheres with 152Cr-labeled microspheres (Dupont-New England Nuclear) labeled with either 14Ce or 55Sr. By using two isotopes, we carried out paired measurements before and after treatments. Because of the anaphylactic response of rats to the commercial dextran vehicle, which resulted in severe hypertension, we modified the protocol by suspending microspheres in 3.5 mol/L glucose, with 0.01% Tween 80 used as an antihypertensive. This concentration of glucose and Tween 80 alone has no effect on systemic pressure. Microspheres at a concentration of 400 000/mL were ultrasonically agitated into suspension for 15 minutes. A volume of 0.2 mL of the suspension, corresponding to 80 000 microspheres, was then drawn into a syringe. The radioactivity within the syringe was counted to obtain the preinjection dose. The syringe was then connected to the left ventricular catheter, and 0.2 mL saline, were infused into the 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 residual postinjection value, and the injection dose was obtained by subtracting preinjection from postinjection counts. To determine the effect of NO synthesis inhibition on renal hemodynamics, microspheres labeled with 55Sr were injected 15 minutes after a bolus dose of 10 mg/kg body wt L-NAME (Sigma Chemical Co). Using this dose, we have found that systemic and renal inhibition of NO synthesis is complete within 10 minutes in both normotensive and hypertensive rats. Under current experimental conditions, higher doses of L-NAME did not increase BP further. Five minutes after the injection of the second set of microspheres, the animals were killed with 150 mg/kg IV Nembutal (Abbott Laboratories). The kidneys were then removed and weighed, and accumulated radioactivity was determined with a Packard gamma counter with dual window settings of 10 to 250 and 400 to 700 meV at a sample level of 0.5 cm. The RBF is expressed in mL · min⁻¹ · g kidney wt⁻¹; RVR, in mm Hg · mL⁻¹ · min⁻¹ · g kidney wt⁻¹ (hereafter referred to as resistance units or RU); CO, in mL · min⁻¹ · 100 g body wt⁻¹; and TPR, in mm Hg · mL⁻¹ · min⁻¹ · 100 g body wt⁻¹ (RU). They were determined as follows: (1) RBF=cpm organ×pump speed×(cpm blood×g kidney wt⁻¹); (2) RVR=mean BP×RBF⁻¹; (3) CO=cpm injected×pump speed×(cpm blood×100 g body wt⁻¹); and (4) TPR=mean BP×CO⁻¹. All results are expressed as the mean±SEM for each group of rats. Changes induced by drug treatment were analyzed with Student’s paired t test. Nonpaired parameters were compared with a standard unpaired Student’s t test. A value of P<0.05 was considered significant. The protocol was approved by our institutional animal care review committee. The experiments were divided into two groups as described below.
Effect of NO Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics in Early- and Chronic-Phase 2K1C Hypertensive Rats

Seven early-phase (4 weeks after clipping) and seven chronic-phase (13 to 16 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 had stabilized, baseline values were obtained by injecting the first set of microspheres as described above.

Thirty minutes after the first set of microspheres were injected, the rats were treated with L-NAME to determine the influence of NO synthesis inhibition on systemic and renal hemodynamics. Fifteen minutes later, after BP had restabilized, the second set of microspheres was administered. After 5 minutes, the rats were killed, the kidneys were excised and weighed, and accumulated radioactivity was determined.

Effect of NO Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics in Chronic-Phase 2K1C Hypertensive Rats After Ang II Blockade

Thirteen to sixteen weeks after being clipped, another seven 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 had stabilized, the rats received 10 mg/kg body wt of the nonagonistic Ang II receptor antagonist losartan (DuP 753, Dupont Corp), and BP was again monitored over 30 minutes. After this second 30-minute period or when BP had stabilized, baseline values were obtained by injecting the first set of microspheres as described above.

Thirty minutes after the first set of microspheres was given, L-NAME was administered to determine the effect of NO synthesis inhibition on systemic and renal hemodynamics after Ang II receptor blockade. Fifteen minutes after L-NAME, after BP had restabilized, baseline values were obtained by injecting the second set of microspheres as described above.

Thirty minutes after the first set of microspheres was given, L-NAME was administered to determine the effect of NO synthesis inhibition on systemic and renal hemodynamics after Ang II receptor blockade. Fifteen minutes after L-NAME, after BP had restabilized, baseline values were obtained by injecting the second set of microspheres 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 had stabilized, baseline values were obtained by injecting the first set of microspheres as described above.

Results

Effect of NO Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics in Early- and Chronic-Phase 2K1C Hypertensive Rats

Early-Phase 2K1C Renovascular Hypertension

Basal BP of acute 2K1C hypertensive rats, 4 weeks after clipping, was 149±3 mm Hg, CO was 24.4±1.7 mL·min⁻¹·100 g body wt⁻¹, and TPR was 6.3±0.5 RU. Changes in BP, CO, and TPR in response to NO synthesis inhibition are shown in Fig 1. L-NAME significantly increased BP by 42±3 mm Hg (P<.001), decreased CO by 44% (to 13.7±0.6 mL·min⁻¹·100 g body wt⁻¹, P<.001), and increased TPR by 122% (to 14.0±0.8 RU, P<.001).

The clipped kidneys of all rats studied were significantly smaller (1.34±0.08 g) than the contralateral kidneys (1.94±0.16 g, P<.001). Basal renal hemodynamics, when corrected for kidney weight, were similar in both nonclipped and clipped kidneys. In the nonclipped kidney, RBF was 4.20±0.40 mL·min⁻¹·g kidney wt⁻¹, and RVR was 38.5±5.6 RU. In the clipped kidney, RBF was 4.50±0.40 mL·min⁻¹·g kidney wt⁻¹, and RVR was 35.4±5.6 RU. The similar (corrected) basal RBF in nonclipped compared with the clipped kidneys of 4-week 2K1C hypertensive rats suggests that these animals had only a mild degree of “functional stenosis,” as defined previously. Changes in RBF and RVR in the nonclipped kidney in response to NO synthesis inhibition are shown in Fig 2, and changes seen in the clipped kidney are shown in Fig 3. In the nonclipped kidney, L-NAME decreased RBF by 20% (P<.01) and increased RVR by 58% (P<.005). In the clipped kidney, L-NAME decreased RBF by 17% (P<.01) and increased RVR by 61% (P<.02). Neither RBF (corrected for kidney weight) nor the percent change from baseline differed between clipped and nonclipped kidneys.

Chronic-Phase 2K1C Renovascular Hypertension

Basal BP of chronic 2K1C hypertensive rats was 166±3 mm Hg. CO was 20.3±1.1 mL·min⁻¹·100 g body wt⁻¹, and TPR was 8.4±0.5 RU. Changes in BP, CO, and TPR in response to NO synthesis inhibition are shown in Fig 1. L-NAME significantly increased BP by 35±6 mm Hg (P<.001), decreased CO by 44% (to 11.3±0.8 mL·min⁻¹·100 g body wt⁻¹, P<.001), and increased TPR by 133% (to 19.6±1.9 RU, P<.001). In chronic-phase 2K1C hypertensive rats, the basal BP was 14 mm Hg higher (P<.001) than in early-phase rats, and TPR was elevated by 33% (P<.01) over basal resistance in early-phase rats. However, there were no
significant differences between early- and chronic-phase rats in the systemic or renal hemodynamic responses to L-NAME.

The clipped kidneys of all chronic-phase 2K1C hypertensive rats studied were significantly smaller (2.04 ± 0.15 g) than the contralateral kidneys (3.63 ± 0.22 g, *P* < .001). Basal renal hemodynamics, when corrected for kidney weight, were similar in both nonclipped and clipped kidneys. In the nonclipped kidney, RBF was 3.50 ± 0.48 mL·min⁻¹·g kidney wt⁻¹, and RVR was 63.6 ± 18.3 RU. In the clipped kidney, RBF was 3.70 ± 0.41 mL·min⁻¹·g kidney wt⁻¹, and RVR was 52.5 ± 10.1 RU. Changes in RBF and RVR in the nonclipped kidney in response to L-NAME are shown in Fig 2, and changes after L-NAME in the clipped kidney are shown in Fig 3. In the nonclipped kidney, L-NAME decreased RBF by 20% (*P* < .01) and increased RVR by 51% (*P* < .005). In the clipped kidney, L-NAME similarly decreased RBF by 17% (*P* < .005) and increased RVR by 60% (*P* < .02). Neither RBF (corrected for kidney weight) nor the percent change in RBF from baseline differed between clipped and nonclipped kidneys. Although basal RVR tended to be higher in the chronic-phase compared with the early-phase 2K1C hypertensive rats, this did not reach statistical significance. There were no differences in the responses to L-NAME between early-phase and chronic-phase 2K1C rats.

**Effect of NO Synthesis Inhibition by L-NAME on Systemic and Renal Hemodynamics in Chronic-Phase 2K1C Hypertensive Rats Pretreated With Losartan**

The basal BP of chronic-phase 2K1C hypertensive rats was 185 ± 8 mm Hg. Acute treatment with losartan decreased BP by only 14 ± 3 mm Hg, to 171 ± 8 mm Hg (*P* < .005). After pretreatment with losartan, CO was 24.5 ± 2.6 mL·min⁻¹·100 g body wt⁻¹, and TPR was 7.8 ± 1.1 RU, similar to the basal values observed in the absence of losartan (Fig 4). Giving L-NAME to losartan-treated rats significantly increased BP by 41 ± 3 mm Hg (*P* < .001), decreased CO by 47% (to 13.0 ± 1.1 mL·min⁻¹·100 g body wt⁻¹, *P* < .005), and increased TPR by 123% (to 17.4 ± 1.8 RU, *P* < .001). All changes induced by L-NAME were similar to those observed in the previously described groups of chronic-phase 2K1C rats that were not treated with losartan.

After treatment with losartan, basal renal hemodynamics (corrected for kidney weight) were similar in both nonclipped and clipped kidneys of chronic-phase 2K1C hypertensive rats. In the nonclipped kidney, RBF was 4.50 ± 0.54 mL·min⁻¹·g kidney wt⁻¹, and RVR was 42.5 ± 5.4 RU. In the clipped kidney, RBF was 4.40 ± 0.76 mL·min⁻¹·g kidney wt⁻¹, and RVR was 54.7 ± 11.8 RU. In chronic-phase 2K1C hypertensive rats, basal RBF and RVR were similar in rats acutely treated with losartan compared with untreated chronic-phase control rats. Changes in RBF and

![Figure 2. Comparison of renal hemodynamics in the nonclipped kidneys between early- and chronic-phase 2K1C hypertensive rats in response to NO synthesis inhibition by L-NAME. Asterisks represent a significant change in response to L-NAME (*P* < .01). There were no significant differences in either basal values or in the response to L-NAME between the early and chronic phase. Values represent the mean ± 1 SE.](http://hyper.ahajournals.org/)

![Figure 3. Comparison of renal hemodynamics in the clipped kidneys between early- and chronic-phase 2K1C hypertensive rats in response to NO synthesis inhibition by L-NAME. Asterisks represent a significant change in response to L-NAME (*P* < .02). There were no significant differences in either basal values or in the response to L-NAME between the early and chronic phase. Values represent the mean ± 1 SE.](http://hyper.ahajournals.org/)
RVR in both nonclipped and clipped kidneys in response to NO synthesis inhibition are shown in Figs 5 and 6, respectively. In the nonclipped kidney, L-NAME decreased RBF by 44% \((P<.005)\) and increased RVR by 110% \((P<.001)\). In the clipped kidney, L-NAME given after losartan decreased RBF by 26% \((P<.05)\) and increased RVR by 76% \((P<.05)\). In the nonclipped kidney of losartan-treated rats, the L-NAME–induced decrease in RBF was significantly greater \((P<.05)\) than the decrease in RBF observed in untreated (chronic-phase) control 2K1C rats (Fig 5). A similar (though nonsignificant) trend in the L-NAME–induced decrease in RBF was also observed in the clipped kidney of losartan-treated rats.

**Discussion**

In rats with long-term or chronic renovascular hypertension, we found that neither the change in BP or CO nor the renal vasoconstriction evoked by acute inhibition of NO synthesis was diminished compared with rats with early-phase 2K1C hypertension. Contrary to what we expected, this suggests that NO still contributes significantly to maintaining vascular tone in chronic 2K1C hypertension and that prolonged hypertension is apparently not exacerbated by developing endothelial dysfunction with diminished NO production. We had hypothesized that this chronic phase of 2K1C hypertension would be characterized by the loss of endothelial function and, therefore, a diminished influence of NO on vascular resistance. On the basis our bioassay of hemodynamic responses to NO synthesis inhibition, neither systemic nor renal endothelial dysfunction are factors in these rats. We further propose that in the chronic phase, blocking the AT₁ receptor would no longer have a profound effect on BP or renal perfusion nor would it retard the response to NO synthesis inhibition. We found that acute administration of the Ang II receptor blocker losartan had only a minimal effect on the underlying hypertension. Furthermore, not only did losartan fail to blunt the renal constrictor response to NO synthesis inhibition, but in the contralateral nonstenotic kidney it also seemed to exaggerate it. We do not have an explanation for why this exaggerated response occurs, and further studies are needed to explain this finding. These data suggest that in this phase of renovascular hypertension, RVR is no longer regulated by the interaction between the dilator tone of NO and Ang II–mediated vasoconstriction as it is in the early phases of the model.\(^{13}\) However, RVR is still significantly influenced by endothelium-derived NO. Presumably, the
vasodilation induced by endogenous NO modifies the effects of constricting factors other than Ang II, such as endoperoxide and/or thromboxane associated with Ang II–dependent hypertension, or other factors, such as physical alterations in resistance vessels.

What is the cause of the dissociation of the balance between NO dilation and Ang II–mediated constriction? Prolonged exposure to elevated Ang II results in an apparent uncoupling of NO release stimulated by shear stress, whereas NO shows enhanced responsiveness to receptor-activated stimulation by factors such as acetylcholine. It has also been suggested that Ang II receptors may be downregulated by chronic exposure to elevated Ang II. Additionally, other constrictor factors, such as endothelium–derived constricting factor, may become more predominant in maintaining the hypertension as the influence of Ang II is diminished. Thus, the dominant role of Ang II in the early phase of renovascular hypertension may be dissipated with chronic hypertension due to multiple factors, including decreased circulating Ang II levels, decreased receptor sensitivity, uncoupling from existing regulatory pathways, and the enhanced influence of alternative constricting factors.

As reviewed by Peach and Loeb, induction of hypertension is associated with endothelial proliferation, but these changes do not occur distal to arterial stenosis, suggesting that they are in response to increased pressure. In contrast, chronic hypertension is characterized by endothelial hypertrophy and changes in the morphology of the tight junctions and gap junctions between endothelial cells, as well as thickening of the basal elastic lamina. It is significant that whereas changes in endothelium–derived prostacyclin may occur shortly after the onset of hypertension, chronic hypertension is characterized by normal prostacyclin synthesis, suggesting that the endothelium has not undergone the significant functional decay thought to be associated with prolonged hypertension. This is consistent with the present study, in which the influence of NO on vascular resistance was apparently maintained.

Unilateral experimental renal artery stenosis producing 2K1C renovascular hypertension is a progressive disease that is characterized by three phases. In phase I (the early phase, from 1 to 5 weeks after clipping), the impaired perfusion of the clipped kidney results in a rise in PRA and circulating Ang II levels and a steady increase in BP to hypertensive levels. In this phase, BP is normalized by the pharmacological blockade of the renin–angiotensin system. In phase II (5 to 8 weeks after clipping), the high PRA levels begin to decline, but the sensitivity to Ang II of the vasculature is increased. BP either remains stable at hypertensive levels or may continue to increase. In this phase, blocking the renin–angiotensin system also normalizes BP, but it takes longer to reach normotensive levels. In phase III (the chronic phase, >9 weeks after clipping), those rats that survive are characterized by reduced levels of renin and circulating Ang II although BP remains elevated. Acute blockade of the renin–angiotensin system has little effect on BP. In our chronic 2K1C population, we found that the systemic depressor response to losartan was only approximately 14 mm Hg; therefore, the rats were still hypertensive after treatment. This is quite different from our previous report on (early-phase) 2K1C hypertensive rats in which Ang II blockade precipitated a rapid 35 to 40 mm Hg drop in pressure to normotensive levels. In that study, pretreatment with losartan also greatly attenuated the vasoconstriction seen after L-NAME in both clipped and nonclipped kidneys while having no effect on the pressor response; this is similar to observations in normotensive rats. The present findings suggest that 13 to 16 weeks after being clipped, surviving rats have reached phase III of 2K1C renovascular hypertension. Although we did not measure PRA in these rats, we have found in a similar group of 20 rats sampled in our laboratory 13 weeks after clipping that PRA was 14 to 16 ng Ang I mL−1 h−1; this is significantly less than the 51 ng Ang I mL−1 h−1 that we reported in early-phase 2K1C hypertensive rats and only slightly higher than the 9 ng Ang I mL−1 h−1 that we found in normotensive rats. This is consistent with previous observations, which reported that after 16 weeks of hypertension, converting enzyme inhibition at similar doses is far less effective in reducing BP than when it is used only 4 weeks after clipping. In contrast, a study by Samani et al found only a slight decrease in PRA during the chronic phase of 2K1C hypertension, but total renin mRNA in the clipped kidney was 42-fold higher compared with the nonclipped kidney, suggesting significant differences in renin synthesis between the two kidneys. Himmelstein and Klotman reported that 16 weeks after clipping, both thromboxane and prostacyclin production are increased in the contralateral kidney,
with the increase in the vasoconstrictor thromboxane B2 being more pronounced. It is not known whether NO plays a role in buffering the systemic constrictor action of endoperoxide/thromboxane B2 in the chronic phase, as it has been shown to in the early phase.20 It is possible that because (endothelium-derived) NO remains an important regulator of TPR in chronic 2K1C hypertension, it may counterbalance the increased influence of thromboxane B2.

We have previously reported that in the early phase of 2K1C hypertension, renal NO plays an important role in regulating systemic and renal hemodynamics by counterbalancing the constrictor influence of elevated circulating Ang II.13 Because persistent long-term hypertension results in vascular damage,18,24 the present study was designed to investigate whether the role played by NO in the regulation of renal hemodynamics is decreased with chronic 2K1C hypertension. Contrary to our hypothesis, we found that NO still plays a major role in regulating systemic and renal hemodynamics. Inhibition of NO synthesis produced changes in systemic and renal hemodynamics similar to those in animals in the early phase of 2K1C hypertension.

We observed that the clipped kidneys of both acute and chronic hypertensive animals were 32% and 45% smaller than their respective nonclipped contralateral kidneys. Interestingly, the RBF (corrected for kidney weight) was equal in the stenotic and contralateral kidneys in all rats studied. We have previously described segregation of rats in the early phase of 2K1C hypertension into three distinct groups based on the ratio of (corrected) RBF in the two kidneys.14 We found that hypertension was more severe and PRA was greater in those rats with reduced RBF in the clipped kidneys. Interestingly, whereas in the present study only 50% of the animals clipped survived to 13 weeks, none of the chronic-phase 2K1C hypertensive rats would be classified as having this more severe functional degree of stenosis with their hypertension. This suggests that when RBF to the clipped kidney does not adapt to a “normal” value (per gram of kidney weight), the animal may not survive to the chronic phase of the disease. Our present data suggest that this survival is related to maintaining the integrity of the endothelium. The responses seen in the clipped kidneys at either 4 weeks or 13 to 16 weeks after clipping showed renal vasoconstrictor responses to NO synthesis inhibition similar to those seen in their respective contralateral kidneys. This is consistent with previous findings in early-phase 2K1C hypertensive rats with mild renal artery stenosis.12 A similar degree of renal vasoconstriction after NO synthesis inhibition in both kidneys of rats with early-phase 2K1C hypertension contrasts with the response seen in early-phase 2K1C hypertensive rats in which the corrected RBF was diminished in the stenotic kidney, implying a more severe (functional) stenosis, and suggests that endogenous renal endothelium-derived NO had diminished influence over renal perfusion.14

In summary, in vivo and in vitro data suggest that various forms of hypertension are characterized by endothelial dysfunction, which may contribute to the rise in BP.1–6 Contrary to these studies, our data suggest that in the chronic phase of 2K1C hypertension the influence of endothelium-derived NO on RVR remains unimpaired. Our results suggest that in the chronic phase of 2K1C hypertension (1) NO acts as an endogenous antihypertensive factor, exerting an important dilator influence that helps maintain renal perfusion despite the increase in TPR, and (2) endothelial dysfunction resulting in decreased NO is not supported by these in vivo data and may not be responsible for sustaining hypertension in this phase of the model. NO appears to remain a potent dilator influence in both systemic and renal hemodynamics in chronic 2K1C renovascular hypertension, apparently counteracting vasoconstrictor influences other than angiotensin.

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