Degree of Renal Artery Stenosis Alters Nitric Oxide Regulation of Renal Hemodynamics

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ABSTRACT

Endothelium-derived nitric oxide (EDNO) maintains RBF in normal kidneys and to the nonclipped kidney of two-kidney, one-clip (2K,1C) renovascular hypertensive rats. However, in clipped kidneys with severe renal stenosis, EDNO has been noted to influence RBF, and it was suggested that low perfusion diminishes the stimulus for shear stress for EDNO synthesis. It was hypothesized that lesser degrees of renal artery stenosis would allow greater renal perfusion and, hence, a greater role for EDNO in maintaining RBF in the clipped kidney. The renal response to EDNO synthesis inhibition was studied with 10 mg/kg body wt N-nitro-L-arginine methyl ester (L-NAME). Four weeks after clipping, rats had different degrees of (functional) renal artery stenosis as determined by the ratio (R) of RBF (per gram kidney weight) in the nonclipped to clipped kidney. Stenosis was classified as either mild (R ≤ 1.25) or moderate (R ≥ 1.30). Both groups were similarly hypertensive (146 ± 3 versus 148 ± 6 mm Hg, respectively) and responded to L-NAME with a 42 mm Hg rise in blood pressure. In 2K,1C rats with mild renal artery stenosis, the renal response to L-NAME was similar in both nonclipped and clipped kidneys. RBF decreased by 17 to 19% (P < 0.005) and renal vascular resistance (RVR) increased by 59 to 63% (P < 0.005). When renal perfusion pressure was controlled, the decrease in RBF was exaggerated 3.6-fold in the nonclipped but only 2.3-fold in the clipped kidney, whereas the RVR increased proportionally. In rats with moderate renal stenosis, L-NAME decreased RBF to the nonclipped kidney by 22% (P < 0.01) and increased RVR by 78% (P < 0.005). However, in the clipped kidney, RBF increased by 51% (P < 0.02), whereas RVR did not change. When renal perfusion pressure was controlled, RBF fell in both kidneys, by 52% in the nonclipped but only 15% in the clipped kidney. The RVR increased by 138 and 21%, respectively. These results suggest that, in 2K,1C rats with only mild stenosis, EDNO is an important regulator of renal perfusion. However, as the apparent degree of renal artery stenosis increases, the influence of EDNO on renal perfusion is diminished.

Key Words: Nitric oxide, renovascular, hypertension, RBF, blood pressure, angiotensin, shear stress, stenosis, endothelium

Since Goldblatt et al. (1) demonstrated experimentally the development of hypertension after renal artery stenosis, the pathogenesis of renovascular hypertension has been studied extensively (2). Clipping the renal artery results in an immediate fall in RBF and GFR in the clipped kidney, whereas plasma renin activity (PRA) and systemic blood pressure (BP) increase (2,3). Within 4 wk, PRA increases 5–10-fold and the rat becomes fully hypertensive (3). In the nonclipped contralateral kidney, renal vascular resistance (RVR) is elevated as the result of an increase in both afferent and efferent arteriolar vasoconstriction (2). However, 4 wk after clipping, both RBF and GFR (per gram of kidney weight) in the nonclipped kidney are similar to those of normotensive controls (2,4), despite elevated BP, RVR, and circulating angiotensin II (AII).

It has been suggested that various forms of hypertension are characterized by a dysfunctional endothelium, resulting in abnormal or diminished endothelium-dependent vasodilation, which may contribute to the rise in BP (5–10). In isolated vessels from two-kidney, one-clip (2K,1C) renovascular hypertensive rats (7), endothelium-dependent vascular relaxation in vitro is impaired but may be restored by reversing the hypertension (7). This suggests that the endothelial dysfunction associated with hypertension may be due to the insufficient production of the intrinsic vasodilator endothelium-derived nitric oxide (EDNO). However, we have previously demonstrated that, during the early phase (4 wk after clipping) of 2K,1C renovascular hypertension, rats with severe renal artery stenosis appear to have an increased role for EDNO synthesis in the nonclipped kidney, perhaps resulting from the increased shear stress of high perfusion, which is a potent endogenous stimulus for EDNO (11). Increased EDNO could counteract the constrictor influence of elevated circulating AII. Conversely, in the severely stenotic kidney, EDNO does not seem to be a factor, because blocking EDNO synthesis did not alter RVR. The diminution of EDNO would allow the vasoconstrictor influence of AII to predominate (12). Thus, EDNO-mediated vasodilation may be a regulatory response that maintains contralateral (nonstenotic) renal perfusion despite ele-
vated. All in this model of hypertension. Increased perfusion of the nonclipped kidney should result in increased vascular shear stress, a primary stimulus for EDNO synthesis (11), whereas in the clipped kidney, stenosis and obstruction of flow presumably will diminish this signal.

Because renal artery stenosis occurs over a spectrum of different degrees of severity in patients with renovascular hypertension, we felt it was important to study subjects with renal artery stenosis less than the severe 80% reduction that we reported previously (12). We hypothesized that, in 2K,1C renovascular hypertension, although EDNO is always an important factor in regulating the perfusion of the nonclipped, contralateral kidney, in the clipped kidney, the degree of renal artery stenosis should dictate the degree of participation of EDNO in regulating renal hemodynamics: lesser stenosis should allow greater renal perfusion, greater shear stress, and hence, a greater influence of EDNO in maintaining RBF. The systemic inhibition of EDNO synthesis results in acute hypertension (13,14), and this response is amplified in hypertensive rats (12,14). If RBF in 2K,1C rats is a balance between RVR and renal perfusion pressure, then the acute exaggerated pressor response seen after blocking EDNO synthesis might obscure the true renal hemodynamic response, especially if there was any degree of endothelial dysfunction in either kidney. Thus, additional experiments were carried out to determine the renal hemodynamic responses in both clipped and nonclipped kidneys after EDNO synthesis inhibition while holding renal perfusion pressure constant at control [pre-N^+-nitro-L-arginine methyl ester (L-NAME)] levels.

**MATERIALS AND METHODS**

All studies were carried out in accordance with institutional guidelines and approved by the Institutional Animal Care Committee. The 2K,1C hypertension was induced as described previously (12). Briefly, male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) weighing 180 to 200 g were anesthetized with sodium pentobarbital (Nembutal; Abbott laboratories, North Chicago, IL). By antiseptic procedures, 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 either 0.20 or 0.23 mm was placed around the renal artery, causing partial occlusion. The wound was closed, and the rat was allowed to recover for 4 wk. Although we initially expected that different size clips should result in different degrees of stenosis, in our hands, neither the degree of stenosis nor the level of hypertension correlated to the size of the clip placed on the renal artery.

The hemodynamic response to EDNO synthesis inhibition by a 10 mg/kg body wt bolus dose of L-NAME (Sigma, St. Louis, MO) was used as an index of the involvement of EDNO in the regulation of systemic and renal hemodynamics. We have previously documented that this dose effectively blocks systemic and renal EDNO synthesis (14). After 4 wk, 2K,1C rats were fasted overnight but were allowed free access to water. They were anesthetized by an ip injection of 125 mg/kg body wt of thiobutabarbital (Inactin; Andrew Lockwood, Milwaukee, WI) and placed on a heating pad to maintain constant body temperature. A PE 10 catheter (Clay Adams, Parsippany, NJ) was introduced through the right common carotid artery and passed into the left ventricle for the infusion of microspheres. The position of the catheter 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. The venous catheter was used for the constant infusion of saline (40 μL/min) and the administration 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 (Viggo-Spectramed, Oxnard, CA) connected to a Gould chart recorder (Gould, Valley View, OHI). After surgery, the rats were allowed a 60-min stabilization period during which BP was monitored.

The bilateral renal hemodynamic response to EDNO synthesis inhibition was measured with radioactive microspheres (15) (DuPont–NEN Research Products, Boston, MA). 15 ± 1.5 μm in diameter, labeled with either 14C or 85Sr. Microspheres were suspended in 3.5 M glucose with 0.01% Tween 80 (an antitaggant) at a concentration of 400,000/mL and mechanically agitated for approximately 15 min. A volume of 0.2 mL of the suspension, corresponding to about 80,000 microspheres, was then drawn up into a syringe. The microspheres, together with 0.2 mL of saline, were infused into the left ventricle over 20 s, while at the same time, arterial reference blood was withdrawn mechanically at a rate of 0.48 mL/min over 75 s. The withdrawn blood was replaced with blood obtained from a donor rat nephrectomized 16 to 24 h earlier. The rats were then allowed to stabilize for 15 min, after which, 10 mg/kg body wt L-NAME was administered to inhibit EDNO synthesis (12). Fifteen minutes after L-NAME administration, a second set of microspheres was injected by the same technique. The animals were then euthanized with an iv injection of Nembutal (150 mg/kg). The kidneys were excised and counted in a Packard gamma counter (Packard Instrument Co., Downers Grove, IL) with dual window settings of 10 to 250 and 400 to 700 keV. In a second series of rats, the renal perfusion pressure was maintained at basal levels after EDNO synthesis inhibition; the aorta was dissected and cleaned at a point superior to the right renal artery, and a constricting loop was placed around it to maintain renal perfusion pressure at pre-L-NAME levels as monitored via the femoral arterial catheter.

RBF (in milliliters per minute per gram kidney weight) was measured as: blood flow = counts per min (cpm) per organ × pump speed (cpm) in blood × kidney weight (in grams). RVR (in millimeters of mercury per milliliter per minute per gram, referred to as resistance units or RU) was determined as: vascular resistance = mean BP/organ blood flow. Cardiac output (CO) (in milliliters per minute per 100 g body wt) was measured as: cpm per injection × pump withdrawal/ (cpm in blood. Total peripheral resistance (TPR) (in millimeters of mercury per milliliter per minute per 100 g body wt; RU) was determined as: mean BP/CO.

Our experimental protocol was designed to test the effect of EDNO synthesis inhibition on regional hemodynamics in 2K,1C renovascular hypertensive rats with either mild or moderate renal artery stenosis. Clipping the renal artery resulted in atrophy of the affected kidney and hypertrophy of the nonclipped contralateral kidney. The absolute (noncorrected) RBF was always reduced in the clipped kidney. However, neither kidney weight nor absolute RBF alone has proved to be a reasonable index of the apparent degree of stenosis. Because we do not have any way of measuring the
actual physical impairment resulting from the stenosis, preventing an anatomical index of the degree of stenosis from being determined, we developed a functional index determined as the ratio (R) of nonclipped to clipped RBF corrected by kidney weight. The corrected ratio was chosen because it represented not only the level of renal perfusion, but it also reflected the relative physical and functional adaptation of each kidney to the clipping. Classification was done during the analysis, after rats had completed the protocols, because neither the aperture of the clip nor any physical parameters were good predictors of the ratio. Rats with \( R \leq 1.25 \) were classified as having mild renal artery stenosis, whereas those with \( R \geq 1.30 \) were classified as having moderate stenosis. The justification for this classification was that an \( R = 1 \) (±25%) suggested only mild stenosis that did not greatly impair (corrected) RBF, whereas higher \( R \) values suggested a greater imbalance such that RBF was more profoundly reduced by the clip. Our theoretical ratios to divide all animals exclusively into two groups were established after we reviewed the data from the initial 10 rats that we had used in the study. No animals were excluded from our classification and analysis. To further assess the degree of stenosis, PRA was measured 15 min after the injection of the second set of microspheres in rats in which renal perfusion pressure was allowed to increase after \( \text{L-NAME} \) administration. The PRA was measured by RIA of angiotensin I (AI) generation by a modification of the technique of Haber et al. (16), as described previously (17). The justification for measuring only post-\( \text{L-NAME} \) PRA was to minimize any changes in basal hemodynamics or altering endogenous AI because of repeated blood sampling (18). As criteria for exclusion, we determined that all rats studied must have: (1) a mean BP after anesthesia that exceeded 140 mm Hg; (2) the clipped kidney must appear to be blood perfused and not necrotic; and (3) the clipped kidney must contain at least 400 microspheres to ensure an accurate determination of flow (15). Six of the rats clipped for this study were eliminated at the time of surgery from further analysis on the basis of these exclusion criteria.

In two groups of experiments, the BP, CO, TPR, renal perfusion pressure, bilateral RBF, and RVR were determined in 2K,1C hypertensive rats, and these rats were later classified as a function of their \( R \) values as having either (1) mild \((N = 5)\), or (2) moderate \((N = 6)\) renal artery stenosis. In the second group of experiments in which renal perfusion pressure was maintained constant at pre-\( \text{L-NAME} \) levels, we also divided rats into groups with either (3) mild \((N = 5)\) or (4) moderate \((N = 5)\) renal artery stenosis. Changes in each parameter induced by \( \text{L-NAME} \) were analyzed by use of a paired \( t \)-test. Changes in different groups were compared by the use of an unpaired \( t \)-test. No multiple testing was used. A \( P < 0.05 \) was considered significant.

RESULTS

Hemodynamics in 2K,1C Rats With Mild Renal Artery Stenosis

In the rats that were classified as having mild renal artery stenosis, the mean \( R \) value (of nonclipped to clipped corrected RBF) was 0.95 ± 0.06. The nonclipped kidneys weighed 1.95 ± 0.14 g and had an uncorrected, absolute RBF of 7.85 ± 0.61 mL/min, whereas the clipped kidneys weighed 1.32 ± 0.07 g and RBF was 5.91 ± 0.54 mL/min. The PRA (taken 15 min after \( \text{L-NAME} \) administration) was 9.0 ± 1.5 ng of AI/mL per hour.

The basal BP of the 2K,1C hypertensive rats classified with mild renal artery stenosis was 146 ± 3 mm Hg, CO was 24.8 ± 1.4 mL/min per 100 g body wt, and TPR was 6.03 ± 0.42 RU. Changes in BP, CO, and TPR in response to EDNO synthesis inhibition are shown in Figure 1A. \( \text{L-NAME} \) significantly increased BP by 42 ± 3 mm Hg \((P < 0.001)\) and TPR by 127% \((P < 0.001)\) while decreasing CO by 43\% \((P < 0.001)\).

Renal hemodynamics in 2K,1C rats with mild stenosis are shown in Figure 2A. The basal (corrected) RBF in the nonclipped kidneys was 4.21 ± 0.39 mL/min per gram kidney weight, similar to the basal RBF of 4.42 ± 0.32 mL/min per gram kidney weight in the clipped kidneys. RVR in the nonclipped kidney was 37.9 ± 4.6 RU and 35.1 ± 4.3 RU in the clipped kidneys. Changes in RBF and RVR in the nonclipped and clipped kidneys in response to \( \text{L-NAME} \) are shown in Figure 2A. \( \text{L-NAME} \) resulted in a similar decrease in RBF (19 versus 17%; \( P < 0.005)\) and an increase in RVR (59 versus 63%; \( P < 0.005)\) in both the nonclipped and clipped kidneys, respectively.

Hemodynamics in 2K,1C Rats With Moderate Renal Artery Stenosis

In the rats classified with moderate renal artery stenosis, the mean \( R \) was 1.81 ± 0.15. The nonclipped kidneys weighed 1.76 ± 0.10 g and had an uncorrected absolute RBF of 7.06 ± 0.67 mL/min, whereas the clipped kidneys weighed 1.29 ± 0.14 g and RBF was 3.21 ± 0.64 mL/min. The PRA was 25.4 ± 0.5 ng of AI/mL per hour.

The basal BP of the 2K,1C hypertensive rats with moderate renal artery stenosis was 148 ± 6 mm Hg, CO was 25.6 ± 2.9 mL/min/100 g body wt, and TPR was 5.99 ± 0.65 RU. All three values are similar to those seen in rats classified with mild stenosis. The responses of BP, TPR, and CO to \( \text{L-NAME} \) are shown in Figure 1A. \( \text{L-NAME} \) significantly increased BP by 42 ± 4 mm Hg \((P < 0.001)\), similar to the change seen in mild stenosis; however, CO did not change, and TPR increased by only 56\% \((P < 0.01)\), less than half the response seen in the mild stenosis rats.

Renal hemodynamics in 2K,1C rats with moderate stenosis are shown in Figure 3A. The basal RBF in the nonclipped kidneys was 4.00 ± 0.20 mL/min per gram kidney weight compared with 2.36 ± 0.26 mL/min per gram kidney weight in the clipped kidneys. The RVR of the nonclipped kidney was 37.1 ± 2.0 RU, compared with 65.5 ± 4.2 RU in the clipped kidneys. Changes in renal hemodynamics in the nonclipped and clipped kidneys in response to \( \text{L-NAME} \) are shown in Figure 3A. In the nonclipped kidneys, \( \text{L-NAME} \) decreased RBF by 22% \((P < 0.01)\) and increased RVR by 78% \((P < 0.005)\). However, in the clipped kidneys, \( \text{L-NAME} \) treatment resulted in an unprecedented 51% increase in RBF \((P < 0.02)\) and no significant change in RVR.
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Figure 1. Changes in systemic hemodynamics in response to inhibition EDNO with 10 mg/kg i-NAME in 2K,1C renovascular hypertensive rats with either mild or moderate renal artery stenosis. Renal perfusion pressure either was allowed to increase after the administration of i-NAME (A) or was maintained constant at pre-i-NAME levels (B). Values are the mean ± SE for each experimental group. Asterisks represent a significant change in response to i-NAME (P < 0.05). bw, body weight.

Hemodynamics With Constant Renal Perfusion Pressure in 2K,1C Rats With Mild Renal Artery Stenosis

In the second group of rats with mild artery stenosis, in which renal perfusion pressure was kept constant, the average ratio of nonclipped to clipped corrected RBF was 1.05 ± 0.07. The nonclipped kidneys weighed 1.62 ± 0.07 g and had an RBF of 7.58 ± 0.07 mL/min, whereas the clipped kidneys weighed 1.16 ± 0.03 g and the RBF was 5.35 ± 0.51 mL/min.

The basal BP of these 2K,1C hypertensive rats with mild renal artery stenosis was 146 ± 3 mm Hg. CO was 21.82 ± 2.5 mL/min/100 g body wt, and TPR was 7.14 ± 0.80 RU. Changes in CO and TPR with controlled renal perfusion pressure are shown in Figure 1B. After the administration of i-NAME, with renal perfusion pressure maintained at 144 ± 3 mm Hg, CO decreased by 49% (P < 0.001) and TPR increased 2.6-fold (P < 0.005). After microphoreses were given, the removal of the constriction loop revealed a systemic pressure of 186 ± 7 mm Hg.

Renal hemodynamics in 2K,1C rats with mild stenosis and controlled renal perfusion pressure are shown in Figure 2B. The basal RBF in the nonclipped kidney was 4.77 ± 0.19 mL/min per gram kidney weight, similar to the 4.60 ± 0.38 mL/min per gram kidney weight in the clipped kidney. The RVR of the nonclipped kidney was 30.6 ± 1.7 RU, similar to 31.5 ± 2.5 RU in the clipped kidney. Changes in RBF and RVR in the nonclipped and clipped kidneys induced by i-NAME at a constant renal perfusion pressure are shown in Figure 2B. In the nonclipped kidney, RBF decreased by 56% (P < 0.001), which was 3.6 times greater than the decrease seen when renal perfusion was allowed to increase; RVR increased by 171% (P < 0.01), also proportionally higher than with uncontrolled perfusion pressure. In the clipped kidney, i-NAME decreased RBF by only 37% (P < 0.005) and increased RVR by 77% (P < 0.05). The decrease in RBF induced by i-NAME in the clipped kidney was 2.3-fold greater than that seen when perfusion pressure was not controlled. However, controlling renal perfusion pressure dissociated the changes in RBF in the two kidneys, so that the decrease in the clipped kidney was only 60% of that seen in the nonclipped kidney (P < 0.005).
Figure 2. Changes in RBF and RVR in both the clipped (stenotic) and nonclipped (contralateral) kidneys of 2K,1C renovascular hypertensive rats, classified as having mild renal artery stenosis, in response to inhibition EDNO with 10 mg/kg i-NAME. Renal perfusion pressure either was allowed to increase after the administration of i-NAME (A) or was maintained constant at pre-i-NAME levels (B). Values are the mean ± SE for each experimental group. Asterisks represent a significant change in response to i-NAME (P < 0.05). kw, kidney weight.

Hemodynamics With Constant Renal Perfusion Pressure in 2K,1C Rats With Moderate Renal Artery Stenosis

In the second group of rats with moderate renal artery stenosis, the average ratio of nonclipped to clipped corrected RBF was 1.41 ± 0.10. The nonclipped kidneys weighed 1.78 ± 0.01 g with an uncorrected RBF of 8.58 ± 0.42 mL/min, whereas the clipped kidneys weighed 1.19 ± 0.07 g and the RBF was 4.14 ± 0.48 mL/min.

The basal BP of the 2K,1C hypertensive rats with moderate renal artery stenosis was 145 ± 2 mm Hg, CO was 26.83 ± 1.22 mL/min per 100 g body wt, and TPR was 5.39 ± 0.39 RU. Changes in CO and TPR with controlled renal perfusion pressure are shown in Figure 1B. After i-NAME, while renal perfusion pressure was maintained at 145 ± 2 mm Hg by aortic constriction, CO decreased by 44% (P < 0.001) and TPR increased 2.4-fold (P < 0.005). The removal of the constricting loop revealed a systemic pressure of 185 ± 4 mm Hg.

Renal hemodynamics in 2K,1C rats with moderate stenosis and controlled renal perfusion pressure are shown in Figure 3B. The basal RBF in the nonclipped kidney was 4.88 ± 0.34 mL/min per gram kidney weight compared with only 3.49 ± 0.38 mL/min per gram kidney weight in the clipped kidney (P < 0.025). The RVR of the nonclipped kidney was 30.2 ± 1.6 RU, compared with 44.1 ± 4.9 RU in the clipped kidney (P < 0.025). Changes in RBF and RVR in the nonclipped and clipped kidneys induced by i-NAME at a constant renal perfusion pressure are shown in Figure 3B. In rats with moderate renal artery stenosis, i-NAME decreased RBF by 52% (P < 0.001) and increased RVR by 138% (P < 0.05) in the nonclipped kidneys. These changes were amplified 2.8 times compared with the changes seen when renal perfusion pressure was not controlled. Further, in the clipped kidney, i-NAME decreased RBF by only 15% (P < 0.005) and increased RVR by only 21% (P < 0.001). These changes were only a third as much as those seen in the nonclipped kidney (P < 0.001). However, the response to i-NAME was now the opposite (decreased rather than increased) of that observed when renal perfusion pressure was not controlled.

DISCUSSION

Renal artery stenosis resulting in renovascular hypertension is a disease of proportion, in that the degree to which renal perfusion is obstructed dictates the development and severity of hypertension (2). In the animal model of this disease (2K,1C renovascular hypertension), clipping one renal artery results in diminished renal perfusion, a rise in systemic pressure, atrophy of the clipped kidney, and an increase in both PRA and circulating ANG II (2). Greater stenosis
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Figure 3. Changes in RBF and RVR in both the clipped (stenotic) and nonclipped (contralateral) kidneys of 2K,1C renovascular hypertensive rats, classified as having moderate renal artery stenosis, in response to inhibition EDNO with 10 mg/kg i-NAME. Renal perfusion pressure either was allowed to increase after the administration of i-NAME (A) or was maintained constant at pre-i-NAME levels (B). Values are the mean ± SE for each experimental group. Asterisks represent a significant change in response to i-NAME (P < 0.05). kw, kidney weight.

compromises renal function and accelerates the pathogenesis of the disease.

EDNO acts as an endogenous antihypertensive factor (11). Numerous in vitro studies of endothelium-dependent vascular relaxation have suggested diminished endothelial function associated with high BP (5–10), implying that the absence of the tonic vasodilator influence of EDNO is permissive for the elevation of BP. We have previously shown an important regulatory interaction between EDNO and AII in maintaining perfusion in the kidneys of normotensive rats (19,20). In hypertension, elevated AII should increase vascular resistance, whereas the rise in BP should increase perfusion of the nonclipped kidney. Each of the factors should result in greater vascular shear stress, a potent stimulus for EDNO synthesis (11).

We have previously reported that in rats with acute (14) or chronic (12) hypertension, the systemic pressor response to the inhibition of EDNO synthesis is not diminished but is actually potentiated. Thus, contrary to the studies evaluating endothelium-dependent vasodilators, our data suggest that EDNO is at least intact if not amplified in response to hypertension. In 2K,1C hypertensive rats with severe renal artery stenosis (with RBF decreased 80 to 90%), EDNO synthesis inhibition also resulted in significantly exaggerated changes in RBF and RVR in the nonclipped kidney (12) compared with previously reported changes in kidneys of normotensive rats (13,14,19,20). These in vivo observations suggest that the endothelium in the contralateral kidney is not dysfunctional and that the role of EDNO is intact, helping to maintain renal perfusion.

Because neither absolute RBF nor the difference in kidney weight has proved to be a good predictor of the degree of renal artery stenosis, we have developed our functional index for an apparent degree of stenosis, determined not on the anatomical obstruction but as the ratio of nonclipped to clipped RBF corrected by kidney weight. Additional assessment of the degree of stenosis was provided by post-i-NAME PRA values. Previously, we reported in normotensive rats that PRA, after EDNO synthesis inhibition, was between 3.7 and 4.0 ng of AI/mL per hour (21). In this study, and consistent with our functional classification of the degree of stenosis, post-i-NAME PRA in rats designated as having mild and moderate renal artery stenosis was more than two times and six times greater, respectively, than that observed in normotensive rats. Additionally, rats with moderate renal artery stenosis had post-i-NAME PRA values that were almost three times greater than that in rats with mild stenosis. In keeping with these results, our previous measurements (unpublished observations) of PRA in rats classified as severe were greater than 50 ng of AI/mL per hour. Thus, we observe that the PRA is elevated,
corresponding to the apparent degree of stenosis, on the basis of our classification.

We have hypothesized that increased stenosis and obstruction of flow should diminish endogenous EDNO. In our previous report with 2K,1C rats, we apparently had severe renal artery stenosis (12). The corrected RBF in the clipped kidney was reduced by 80 to 90% (R = 7.8). Also, inhibiting EDNO synthesis did not induce any significant changes in either RBF or RVR in the clipped kidney. These results are in keeping with our hypothesis, in that the markedly reduced perfusion resulted in EDNO apparently no longer contributing to renal vascular tone. Presumably, the elevated intrarenal and circulating AI in this kidney predominates in the regulation of perfusion. It seemed to us that if nonclipped (high-perfusion) kidneys were highly dependent on EDNO, whereas severely stenotic kidneys were refractory to EDNO, then lesser degrees of stenosis should reflect intermediate levels of EDNO modulation of RBF to the clipped kidney. We hypothesized that the lesser the obstruction of perfusion, the greater the role of EDNO in maintaining RBF, whereas EDNO should remain an important influence on RBF to the nonclipped kidney.

In rats classified with mild renal artery stenosis, EDNO synthesis inhibition resulted in a similar decrease in RBF and an increase in RVR in both nonclipped and clipped kidneys. Although this initially suggested no diminution of EDNO in the vasculature of the clipped kidney, the systemic pressor response was twice that seen in response to L-NAME in normotensive rats (19,20). It has previously been reported that RBF autoregulation is diminished in 2K,1C rats (22); thus, the increased perfusion pressure could alter the renal hemodynamic response to the inhibition of EDNO synthesis. We undertook the same experiment with the added constraint of maintaining constant renal perfusion pressure despite the increase in systemic pressure after L-NAME. Again, RBF decreased and RVR increased in both kidneys, but the decrease in RBF was exaggerated, as predicted (22). Furthermore, the decrease in RBF in the clipped kidney was only 60% of that in the nonclipped kidney. These data suggested that, although there was no overall endothelial dysfunction, the role of EDNO in maintaining RBF in the clipped kidney of rats with mild stenosis was partially diminished, as we had hypothesized.

In rats classified with moderate renal artery stenosis, the results were even more intriguing. L-NAME evoked an exaggerated 42 mm Hg systemic pressor response, again suggesting no systemic diminution of EDNO. As before, the nonclipped kidney exhibited decreased RBF and increased RVR. However, the clipped kidney responded to L-NAME with an unexpected increase in RBF, suggesting that perfusion pressure was driving flow. These studies were repeated in rats with moderate stenosis in which renal perfusion pressure was maintained consistent after L-NAME. As expected, controlling perfusion pressure accentuated the decrease in RBF in the nonclipped kidney and now decreased RBF in the clipped kidney. As hypothesized, the nonclipped kidney appeared to be highly dependent on the influence of EDNO, which in the clipped kidney was greatly attenuated compared with that seen in either the nonclipped or mildly stenotic clipped kidneys.

For the sake of discussion, we have summarized these findings and our previously published observations (12,19) in Figure 4. The nonclipped kidney demonstrates similar or exaggerated responses to EDNO synthesis inhibition, regardless of the classification, compared with the responses we have reported in normotensive rat kidneys (upper panel). In contrast, in the clipped kidney, as the apparent degree of stenosis progresses from none to severe, the response to L-NAME is diminished (lower panel). This suggests that the influence of EDNO in maintaining renal perfusion decreases in the clipped kidney as the relative stenosis becomes greater, similar to what we have hypothesized. Overall, these observations suggest that EDNO plays an important role in maintaining perfusion of the nonclipped kidney of rats with renal artery stenosis but is progressively diminished in the clipped kidney. We have assumed that an anatomical stenosis parallels our functional classification and
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have hypothesized that increasing the obstruction to perfusion results in reduced shear stress, reduced stimulus for EDNO, and a shift in the balance from EDNO vasodilatation to AI-mediated renal vasoconstriction. Consistent with this is a report that the repair of renal artery stenosis results in a rapid reversal of hypertension and a normalization of RBF, both of which are blocked by previously inhibiting EDNO synthesis (23).

Although many factors can stimulate the production of EDNO, shear stress may be the most important physiologic stimulus. It has been hypothesized that flow-induced changes in vascular tone may be mediated by conformational changes in flow sensors, resulting in changes in sodium and calcium binding that in turn influence vascular smooth muscle tone (24). Flow-induced vasodilatation may be mediated through the calcium-dependent production of EDNO (24). We have not directly determined shear stress in our studies, and we make the presumption that, as the stenosis becomes more severe, limiting renal perfusion, shear stress will diminish. This study supports the concept that EDNO is an important signaling factor of flow-mediated changes in vascular tone.

First, clipped kidneys, which presumably have impaired blood flow, showed an attenuated decrease in RBF after L-NAME compared with the nonclipped, contralateral kidneys. Second, absolute RBF in the clipped kidney was 32% higher in mild than in moderate renal artery stenosis. Further, the L-NAME-induced decrease in RBF was 54% less in kidneys with moderate compared with mild stenosis, whereas in our previous report (12), severe stenosis totally eliminated the RBF response. These findings strongly suggest that the degree of (functional) renal artery stenosis dramatically alters the influence of EDNO on renal perfusion.

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 (25). Thus, although we are using an AI-dependent model of hypertension, the exaggerated systemic pressor response we observed after L-NAME may be a function of elevated systemic resistance in hypertension but is not necessarily due only to AI. The exaggerated pressor response to L-NAME also implies that, overall, the endothelium is not dysfunctional in 2K,1C hypertension (4 wk after clipping), as previously suggested by in vitro studies (7), but rather may serve as an important buffer countering hypertensive influences.

In conclusion, our data suggest that in rats with 2K,1C renovascular hypertension, EDNO synthesis (or its apparent influence) is increased both systemically and in the nonclipped kidney, presumably because of the positive signal of increased vascular shear stress, elevated pressure, and AI. Conversely, in the clipped kidney, the influence of EDNO on renal perfusion is reduced, apparently related to the (functional) degree of renal artery stenosis. We hypothesize that the degree of EDNO’s influence is related to the extent of shear stress, which may serve as the local regulator of EDNO synthesis. Thus, as renal artery stenosis progresses to greater severity, there is diminished perfusion, less EDNO-mediated vasodilator tone, and elevated shear and circulating angiotensin. It is likely that this combination further constrains the kidney, leading to progressively less renal perfusion and function and, ultimately, the acceleration of the disease.

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