Nitric Oxide Dependence of Renal Blood Flow in Patients with Renal Artery Stenosis

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Abstract. In ischemia, nitric oxide (NO) production is increased, possibly to preserve flow. The role of NO was investigated in hypertensive patients with or without renal artery stenosis (RAS). Fifty-five hypertensive patients scheduled to undergo diagnostic renal angiography underwent mean renal blood flow (MRBF) measurements before and after an intrarenal injection of the NO synthase blocker N^G-monomethyl-L-arginine (L-NMMA) at 0.03 μg/kg, before angiography. A dose-response study indicated that this dose of L-NMMA significantly blocked NO synthesis. MRBF was measured at baseline and 1, 5, 10, and 20 min after L-NMMA treatment. On the basis of the angiographic results, patients were divided into three diagnostic categories, i.e., essential hypertension (n = 26), unilateral RAS (n = 16), or bilateral RAS (n = 8). In essential hypertension, MRBF was decreased by 18 ± 4% at 20 min. In unilateral RAS, L-NMMA did not affect MRBF in the stenotic kidney but reduced MRBF in the nonstenotic kidney by 40 ± 9% at 20 min. In bilateral RAS, L-NMMA reduced flow by 32 ± 14% at 20 min. In the nonstenotic kidney in unilateral RAS, a positive correlation was observed between the effect of NO blockade on MRBF and arterial renin levels (P = 0.009). In the stenotic kidney, in contrast, this correlation was inverse (P = 0.007). In conclusion, MRBF depends on NO in hypertensive patients, except in the stenotic kidney in unilateral RAS. In the nonstenotic kidney in unilateral RAS, NO bioavailability is increased. It is suggested that a compensatory mechanism, regulated by NO and possibly angiotensin II, may preserve renal function.

Nitric oxide (NO) is an important regulator of vascular tone and tissue perfusion. NO production is increased under ischemic conditions, as demonstrated, for example, in coronary arteries (1,2). This increase could be considered an adaptive mechanism to preserve flow as much as possible. In intact human subjects, renal artery stenosis (RAS) represents a clinical condition in which renal ischemia develops progressively with time. This is associated with stimulation of the renin-angiotensin system and postglomerular vasoconstriction to maintain the GFR (3). However, angiotensin II (AngII) also upregulates NO production, possibly to protect the kidney against further (preglomerular) ischemia (4). Indeed, recent reports indicate that increased levels of AngII enhance the production of endothelial NO synthase (eNOS) (5,6). This finding has been corroborated in animal studies, which have demonstrated that NO levels are increased in both the acute (7) and chronic (8) phases of ischemia, particularly in nonstenotic kidneys (9). The latter findings suggest that the contralateral kidney is protected from AngII-induced vasoconstriction. On the basis of these data, we hypothesized that, in human subjects with RAS, NO bioavailability is increased to maintain renal perfusion. Therefore, the aim of our study was to assess the role of NO in the regulation of renal blood flow in hypertensive patients with or without RAS.

Materials and Methods

Patients

This study was performed with 55 hypertensive patients for whom RAS was suspected on the basis of one or more of the following criteria: persistent hypertension despite the use of at least three antihypertensive agents, overt peripheral vascular disease, the presence of an abdominal bruit, or an increase in serum creatinine levels after angiotensin-converting enzyme inhibitor treatment. Patients were asked to participate only after the indications for angiography were apparent. Three weeks before the procedure, administration of all antihypertensive medications was discontinued. In addition, patients were asked to follow a salt-restricted diet (55 mmol of sodium/d) during the last week before admission. The rationale for the latter is that data on renal blood flow should always be interpreted in relation to sodium intake and standardization is therefore mandatory. We chose an amount of 55 mmol/d because this agrees with our previous studies. In our experience, this degree of sodium restriction provides the best discrimination among patients with low, normal, and high renin levels. Adherence to the diet was confirmed by measurement of 24-h sodium output at the time of admission to the hospital. Patients were also instructed not to smoke or use any caffeine-containing products for at least 48 h before the investigations.
Experimental Procedures

The experimental procedure was performed in the angiographic suite of the department of radiology, which is equipped with an x-ray system and a gamma camera. After selective catheterization and before administration of contrast material, blood samples for determination of active plasma renin concentrations (APRC) and AngII levels were drawn simultaneously from the aorta and both renal veins. The mean renal blood flow (MRBF) was subsequently assessed, by means of the xenon washout technique (10), first in the left kidney and then in the right kidney. After baseline data had been obtained, intervention studies (see below) were initiated, using $N^\omega$-monomethyl-l-arginine ($\omega$-NMMA) to block intrarenal NO synthase (NOS) activity. For reasons of standardization, these studies were always performed in the right kidney. Heart rate and intra-arterial BP were monitored continuously during each MRBF measurement. Angiography was performed only after all measurements had been completed. Angiographic films of the abdominal aorta and renal arteries were obtained in the anteroposterior and two oblique directions, using a 5-French pigtail catheter positioned at the level of the renal arteries and 45 ml of nonionic contrast medium for each view. If the renal arteries were not adequately depicted, selective renal angiography was performed with an end-hole catheter.

All patients gave written informed consent, and the study was approved by the Maastricht University Hospital Medical Ethics Committee.

Angiographic Evaluation

Immediately after the procedure, angiographic films were evaluated by a radiologist who was unaware of the results of the blood flow measurements. Patients were classified as having either no abnormalities of the renal arteries or unilateral or bilateral RAS, depending on the absence or presence of visible abnormalities in the plain-view angiograms. Subsequently, stenoses were graded in zoomed images, using an electronic caliper with 0.1-mm accuracy. Reductions in luminal diameter were calculated using two-dimensional coronal plain views of the renal arteries. As demonstrated earlier, the intra- and interobserver variabilities for this type of measurements averaged 11 and 12%, respectively (11). Because of the difficulty of obtaining objective measurements of stenosis for patients with fibromuscular dysplasia, no attempts were made to calculate the degree of stenosis for these patients.

Study 1: Dose-Response Relationship for L-NMMA

To establish the dose of L-NMMA necessary to inhibit NOS, 15 patients were randomly allocated to receive a bolus injection of either 0.01, 0.03, or 0.1 $\mu$g/kg L-NMMA, administered into the renal artery within 10 s. MRBF was measured 1, 5, 10, and 20 min after bolus injection. To preclude confounding of the results by renal vascular abnormalities, only patients with normal angiographic findings were selected for analysis of these data.

Study 2: Effect of NOS Inhibition on MRBF

On the basis of the results of study 1, bolus injections of 0.03 or 0.1 $\mu$g/kg L-NMMA were administered intrarenally to another 33 and seven patients, respectively. For all of these patients, MRBF was measured at baseline and 1, 5, 10, and 20 min after the intra-arterial bolus injection. Patients who had been included in the dose-response study (study 1) and who had received either 0.03 or 0.1 $\mu$g/kg L-NMMA were also included in the analysis of study 2.
three doses used. In all groups, L-NMMA produced a significant decrease in MRBF within 5 min. In the group that received 0.01 μg/kg, MRBF returned to baseline levels 5 to 15 min after a maximal decrease of 12 ± 21%. At 20 min, there appeared to be vasodilation. In contrast, MRBF plateaued after 10 min in both other groups, with the plateau lasting until 20 min after the injection of L-NMMA. The reductions in flow at 10 min were 19 ± 9% and 24 ± 15% after doses of 0.03 and 0.1 μg/kg, respectively. Although the effects were reached slightly earlier with the 0.1 μg/kg dose than with the 0.03 μg/kg dose, differences were not otherwise statistically significant. BP and heart rate did not change during any of the studies.

**Study 2**

**Baseline Data.** Baseline data for MRBF are presented in Table 2. Because the effects of 0.03 and 0.1 μg/kg L-NMMA on MRBF were not significantly different, data were pooled for further analysis. BP and heart rate did not change during any of these experiments.

**EH.** For patients with EH (n = 26), intrarenal injection of L-NMMA induced a significant decrease in MRBF (P = 0.007) (Figure 2). At 20 min, the decrease in renal blood flow amounted to 18 ± 4%. The mean IVR was −247 ± 61 U (P = 0.001, compared with baseline values).

**Unilateral Stenosis.** For patients with unilateral stenosis (n = 16), the degree of stenosis ranged from 15 to 95% (Table 1). Although baseline MRBF tended to be lower in stenotic kidneys than in nonstenotic kidneys, the difference was not significant (110 ± 19 ml/min per 100 g versus 163 ± 24 ml/min per 100 g).

**Figure 1.** Dose-effect relationship. Percent changes in mean renal blood flow (MRBF) are plotted against measurement times. Bolus injections of three different doses of Nω-monomethyl-L-arginine (L-NMMA) were used (■, 0.01; ▲, 0.03; ●, 0.1 μg/kg). Data are presented as median values.

| Table 1. Patient characteristics at baselinea |
|-------------------|-------------------|-------------------|
|                   | EH                | Unilateral RAS    | Bilateral RAS   |
| No. of patients   | 26                | 16                | 8               |
| Age (yr)          | 51 ± 2            | 56 ± 3            | 59 ± 5          |
| Sex (F/M)         | 11/15             | 3/13              | 4/4             |
| BMI (kg/m²)       | 27.7 ± 1          | 24.7 ± 0.5        | 27.4 ± 1.7      |
| MAP (mmHg)        | 132 ± 4           | 129 ± 6           | 137 ± 8         |
| HR (beats/min)    | 73 ± 3            | 71 ± 4            | 72 ± 3          |
| 24-h urine volume (ml) | 1718 ± 119       | 1513 ± 156        | 1744 ± 129      |
| 24-h urinary sodium excretion (mmol) | 93 ± 10           | 71 ± 10           | 84 ± 12         |
| Arterial APRC (mU/L) | 22 ± 3            | 37 ± 12           | 34 ± 7          |
| Venous APRC, stenotic kidney (mU/L) | 26 ± 4            | 40 ± 11           |                |
| Venous APRC, nonstenotic kidney (mU/L) | 39 ± 16           | 9 ± 3             |                |
| APRC ratio, stenotic kidney | 15 ± 4            | 18 ± 7            |                |
| APRC ratio, nonstenotic kidney | 13 ± 1            | 18 ± 3            | 21 ± 4          |
| Arterial angiotensin II level (pM)b | 25 (15–37)         | 46 (28–56)        |                |
| Degree of stenosis, left kidney (%)b | 53 (27–95)         | 25 (13–40)        |                |
| Degree of stenosis, right kidney (%)b |                    |                   |                |

**Table 2. MRBF at baseline in patients with EH or bilateral or unilateral RASa**

<table>
<thead>
<tr>
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<th>Baseline MRBF (ml/min per 100 g)</th>
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<tr>
<td></td>
<td>Left/Stenoticb</td>
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<tr>
<td>EH (n = 26)</td>
<td>158 ± 16</td>
</tr>
<tr>
<td>Bilateral RAS (n = 8)</td>
<td>137 ± 32</td>
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<tr>
<td>Unilateral RAS (n = 16)</td>
<td>110 ± 19d</td>
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a For explanation, see text. Data are presented as means ± SEM. MRBF, mean renal blood flow.
b For unilateral RAS.
c P = 0.018 for difference versus the stenotic kidney in unilateral RAS.
d P = 0.035 for difference versus EH.
ml/min per 100 g; 0.05 < P < 0.10). Baseline MRBF in stenotic kidneys was significantly lower than that in EH (P = 0.035). L-NMMA was injected into the stenotic kidney of seven patients and the nonstenotic kidney of nine patients. On average, MRBF in stenotic kidneys did not change significantly after bolus injection of L-NMMA (Figure 2). The mean IVR in these kidneys was +34 ± 149 U (not different from baseline). In contrast, MRBF in nonstenotic kidneys decreased by 40 ± 9% at 20 min, with a mean IVR of −751 ± 142 U (P = 0.008), after L-NMMA injection. Pairwise comparisons of IVR between stenotic and nonstenotic kidneys demonstrated a significant difference (P = 0.002) (Figure 3). Compared with the response in EH, nonstenotic kidneys of patients with unilateral RAS exhibited a significantly greater response to L-NMMA (P = 0.001).

Bilateral Stenosis. In this group, the degree of stenosis ranged from 28 to 56% in the left kidney and from 16 to 40% in the right kidney (Table 1). Baseline MRBF in this group was significantly higher than that in the stenotic kidneys of patients with unilateral RAS (P = 0.018) but was not higher than that in the other groups (Table 2). In patients with bilateral RAS, MRBF was reduced by 32 ± 14% at 20 min after L-NMMA administration. The mean IVR of −590 ± 218 U was significantly different from baseline (P = 0.018) (Figure 2). The IVR was significantly greater than the IVR in the stenotic kidneys of patients with unilateral RAS (P = 0.048) but was not greater than that in EH or in nonstenotic kidneys of patients with unilateral RAS (Figure 3).

Correlations with Renin and AngII Levels and the Degree of Stenosis. For patients with unilateral RAS, correlations were observed between the effects of L-NMMA on MRBF (IVR) and arterial APRC (Figure 4). The correlation between IVR and arterial APRC was inverse in the stenotic kidneys (P = 0.007), whereas there was a positive correlation in the nonstenotic kidneys (P = 0.009). The IVR was also inversely related to arterial AngII levels in the stenotic kidneys (P < 0.001), but the relationship between AngII levels and IVR in the nonstenotic kidneys just failed to reach statistical significance (P = 0.058). No correlations between APRC or AngII levels and IVR were observed for the other groups.

When patients with RAS of >50% were compared with those with milder degrees of stenosis, baseline MRBF appeared to be lower in the former (median values of 45 versus 156 ml/min per 100 g). Because of the large splay of the data and the relatively small number of patients in each group, this difference failed to reach statistical significance. Although we observed no relationship between the IVR and the degree of stenosis in any of the groups, a complete lack of change in MRBF after intrarenal administration of L-NMMA was confined to patients with unilateral stenosis of <50%.

Discussion

This study demonstrates that, with the doses of L-NMMA used, it was possible to reduce intrarenal NO synthesis in human subjects without producing systemic effects. The results of the first experiment indicated that L-NMMA caused a dose-related decrease in renal blood flow within minutes after intrarterial bolus injection. This initial vasoconstriction rapidly waned with the lowest dose (0.01 µg/kg), even demonstrating rebound vasodilation. However, from 10 to 20 min, sustained vasoconstriction was observed with the middle dose (0.03 µg/kg) and the highest dose (0.1 µg/kg) of L-NMMA; there was no difference in effect between the two doses. These data suggest that an intrarenal bolus injection of L-NMMA immediately blocks intrarenal NO synthesis, which is followed by complete or partial escape. Whether this escape is attributable to recovery of NOS activity or to compensatory stimulation of vasodilatory mechanisms cannot be determined from our data.

In the second part of our study, we observed that, in EH, resting renal blood flow was partly dependent on NO. In unilateral RAS, however, NO bioavailability was absent in stenotic kidneys with low-grade stenosis but increased in nonstenotic kidneys, compared with that in kidneys of patients with EH. In subjects with bilateral RAS, NO availability was...
increased in both kidneys, in comparison with stenotic kidneys of patients with unilateral RAS. Unfortunately, we cannot comment on the role of intrarenal NO in normotensive subjects, because such subjects could not be included in our study for obvious reasons.

Using L-NMMA, other investigators also observed NO-dependent flow in different vascular beds in patients with EH, although responses to the blocker were blunted, compared with normotensive control subjects (18). Dijkhorst-Oei et al. (19) reported a decrease in renal blood flow of 58% in patients with EH after systemic inhibition of NOS by L-NMMA, a procedure that also increased BP. In their study, renal blood flow was measured by clearance methods. In our patients with EH, MRBF was decreased by approximately 18% after local NOS inhibition but, because we measured flow per unit mass of renal tissue with a marker that does not use tubular transport mechanisms, our data and theirs are difficult to compare. Moreover, by design, our study allowed evaluation of renal vascular responses in the presence of unchanged arterial pressure.

In unilateral RAS, the NO dependence of MRBF in the nonstenotic kidney could represent a compensatory mechanism to preserve net GFR. This contention is supported by data obtained in animal experiments. For instance, in a model of renovascular hypertension, Bosse and Bachmann (20) demonstrated nitrotyrosine (which is very indicative of NO production) in the arteriolar walls of the nonclipped renal artery, whereas nitrotyrosine was exclusively present in the extraglomerular mesangium in the clipped kidney. NOS has quite different functions, depending on its localization (21). Whereas eNOS is stimulated by an increase in renal blood flow (or shear stress on the arterial wall), neuronal NOS (nNOS) is stimulated when renal blood flow decreases (with activation of the tubuloglomerular feedback system). The responses of eNOS are fast and short-lasting, but those of nNOS are slow and long-acting. Although the ultimate goal of the two systems is the same (recovery of renal function), their mechanisms of action seem to be opposite. Our results are compatible with inhibition of eNOS, although we cannot exclude the possibility that nNOS was also inhibited. Moreover, on the basis of the data of Bosse and Bachmann (20) for the clipped kidneys of two-kidney/one-clip rats, we must leave open the possibility that there is a problem with the degradation of NO, rather than with its generation.

Earlier studies demonstrated that, despite elevated BP, renal vascular resistance, and circulating AngII levels in two-kidney/one-clip rats, renal blood flow and GFR were comparable to those in normotensive control animals (22,23). These data support the idea that compensatory vasodilation occurs in the nonstenotic kidney. This hypothesis was also evaluated by Sigmon and Beierwaltes (24). Under conditions of controlled perfusion pressure (no systemic effects, as in our model), those authors observed a greater decrease in renal blood flow in the nonstenotic kidney, compared with the stenotic kidney, in both mild and moderate degrees of stenosis (24). Our results in unilateral stenosis are in line with their data. Recently, Turkstra et al. (25,26) confirmed these observations with respect to

Figure 4. Correlations in unilateral RAS. (A and C) Stenotic kidneys. (B and D) Nonstenotic kidneys. Correlations between the effects of L-NMMA on MRBF (IVR) and arterial (art) active plasma renin concentrations (APRC) (A and B) and angiotensin II (AngII) levels (C and D) were observed. Correlation coefficients and P values are noted. The dotted lines represent the 95% confidence interval of the IVR in EH. A negative IVR indicates the presence of nitric oxide synthesis (vasoconstriction after L-NMMA injection).
tubuloglomerular feedback in two-kidney/one-clip rats. In two-
other vasodilators seem
In bilateral RAS itself cannot be the only factor responsible for altered NO production, because then a similar increase in NO production in the stenotic kidneys to maintain GFR as long as possible (32).
In contrast to the nonstenotic kidney, apparently no tendency for NO-mediated vasodilation is operative in the stenotic kidney. The lack of such a compensatory mechanism may initially seem odd. However, from a hemodynamic point of view, it could be argued that poststenotic vasodilation is undesirable because it enhances the stenotic effect and may further decrease perfusion pressure (32). In fact, this may also be the reason why increased AngII levels induce postglomerular vascondstriction, because this offers the best conditions for stenotic kidneys to maintain GFR (32).
In unilateral RAS, there is no possibility for compensation such as that in unilateral RAS. Our data suggest that, when both renal arteries become stenotic, the endothelium of both kidneys must produce NO to maintain optimal GFR. This would explain the marked vasoconstrictor response to L-NMMA, suggesting high NO bioavailability in both kidneys of these patients. Dubey et al. (33) observed that NO synthesis was increased, at least in the early phase, in one-kidney/one-clip hypertensive rats. Although this can hardly be compared with our human model, the data suggest that RAS itself cannot be the only factor responsible for altered NO production, because then a similar increase in NO production in the stenotic kidneys of patients or animals with unilateral stenosis would be expected. Unfortunately, not much is yet known regarding endothelial function in unilateral RAS.
Our finding of an inverse correlation between arterial APRC and AngII levels and the effects of L-NMMA in stenotic kidneys, with a positive correlation in nonstenotic kidneys, is remarkable. Higher APRC and AngII levels were correlated with greater NOS activity in stenotic kidneys, whereas higher APRC levels were correlated with decreased NOS activity in nonstenotic kidneys. These findings agree well with reports by Sigmon and co-workers (4,34) and by Qiu and Baylis (35), who stated that AngII mediates most glomerular responses to NOS inhibition. To interpret these opposing relationships in the stenotic and nonstenotic kidneys, one must look for different mechanisms at the tissue level, because systemic effects would induce comparable responses in the two kidneys. These mechanisms could be related to different levels of expression of AngII receptors in the kidneys (32,36) and/or to different densities of angiotensin subtype 2 receptors (37–39).
Obviously, there are a number of limitations in our study. First, not all lesions in our patients with RAS met the criteria for hemodynamically significant stenosis, as defined, for example, in intervention studies (40). Clinically relevant stenoses, however, were not the prior target of our study. The mere presence of even minor renovascular abnormalities seems to affect the regulation of renal blood flow. This was suggested earlier by Schreij et al. (41), who demonstrated that renalographic changes can be detected with 30% arterial narrowing. This finding does prompt the question of whether invasive therapeutic measures such as PTRA or stenting should be considered at earlier stages of the disease. Second, with respect to endothelial function, we reduced NO synthesis in only one kidney per patient. Therefore, it is not possible to compare, in individual patients, the effects of NO inhibition in both kidneys. Third, we reduced only NO synthesis and, because MRBF is influenced by both vasoconstrictors such as endothelin (42) and vasodilatory factors, we studied a net effect, without taking into account compensation by other factors. It is also unclear which forms of NOS are inhibited by bolus injections of L-NMMA and whether continuous infusions of L-NMMA would yield different results. Another point to be considered is that we studied our patients while they were achieving balance with a mildly sodium-restricted diet. Therefore, we are not certain whether similar results would have been obtained with normal or high sodium intake. Also, our method of measuring renal blood flow requires scrutiny. Indeed, when renal function is compromised, distinction between the fast and slow components may no longer be possible. With the data presented here, however, this was not a frequent problem. Finally, there may be differences in endothelial function among hypertensive patients, depending on their previous medical treatment (43). However, because of the washout period of 3 wk, we consider the latter to be of minimal importance.
In summary, this study shows that, in human hypertension, renal blood flow is dependent on NOS activity in the renal vasculature. In unilateral renovascular hypertension, MRBF is no longer NO dependent in the stenotic kidney, as opposed to the nonstenotic renal artery, in which MRBF is strongly NO dependent. This effect is apparent even with low-grade stenosis. We hypothesize that, in human unilateral renovascular hypertension, a compensatory mechanism develops to maintain optimal renal function. This compensatory mechanism is regulated by NO and probably also by AngII. Finally, our study also demonstrates that endothelial function, inasmuch as it is reflected by NO synthesis, can exhibit regional differences even within one organ system.

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