Nitric Oxide Modulates Vascular Tone in Preglomerular Arterioles

John D. Imig and Richard J. Roman

Blockade of nitric oxide reduces renal blood flow, but the site or sites at which nitric oxide alters renal vascular resistance are unknown. The effects of N^ω-nitro-L-arginine (100 μM), an inhibitor of nitric oxide synthesis, on the pressure–diameter relation of renal arterioles was studied using a rat juxtamedullary microvascular preparation perfused in vitro with a physiological salt solution containing 5% albumin. The basal diameters of the main arcuate and interlobular arteries and the proximal and distal afferent arterioles averaged 438±26, 64±4, 30±1, and 20±1 μm, respectively, at a perfusion pressure of 80 mm Hg. The diameters of the arcuate and interlobular arteries increased by 14 ±2% and 7 ±2%, whereas the proximal and distal afferent arterioles decreased by 3±1% and 7±2% when perfusion pressure was elevated to 160 mm Hg. Nitro-arginine had no effect on the basal diameters of arcuate and interlobular arteries. Nitro-arginine reduced the diameters of afferent arterioles by 7 ±2% at all perfusion pressures studied. Nitro-arginine increased active vascular tone in the interlobular artery and afferent arterioles and enhanced autoregulation of glomerular capillary pressure. L-Arginine (1 mM), the precursor to nitric oxide production, reversed the effects of nitro-arginine. These findings suggest that nitric oxide modulates vascular tone of the interlobular artery and afferent arterioles of deep nephrons and influences the ability of the preglomerular vasculature to autoregulate glomerular capillary pressure. (Hypertension 1992;19:770–774)

KEY WORDS • nitric oxide • endothelium • autoregulation • kidney • microcirculation • renal circulation

Vascular endothelial cells release an endothelium-derived relaxing factor, which has identical properties as nitric oxide (NO). NO is thought to be tonically released from the endothelium and influences vascular tone. The vasodilator and diuretic responses to intrarenal infusion of the endothelium-dependent vasodilators acetylcholine and bradykinin are dependent on NO release. Recently, the renal circulation has been shown to be especially sensitive to inhibition of NO production. Renal blood flow and to a lesser extent glomerular filtration rate are reduced after administration of N-nitro-arginine or N-monomethyl-L-arginine; however, the sites in the renal circulation at which NO influences vascular tone have not been identified. The present study examined the effects of inhibition of NO production on pressure–diameter relations in the renal circulation using the juxtamedullary nephron microvascular preparation perfused in vitro with a cell-free physiological salt solution (PSS) containing 5% albumin.

Methods

Experiments were performed on 22 Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) weighing an average of 327±13 g. The rats were anesthetized with pentobarbital (65 mg/kg body wt i.p.), and the left kidney was isolated and perfused for study of the juxtamedullary vasculature according to the method of Casellas and Navar as modified by Sanchez-Ferrer et al. The kidney was perfused with cell-free PSS as previously described that was supplemented with a mixture of L-amino acids (except L-arginine) to support tubular function. Perfusion pressure was continuously monitored with a pressure transducer. The kidney was removed from the rat, placed in an organ chamber, and superfused with PSS that was equilibrated with 95% O_2–5% CO_2 (pH 7.4).

The isolated vascular segment was pressurized to 80 mm Hg, and 0.02% fluorescein isothiocyanate-labeled γ-globulin was added to the perfusate to allow the vascular diameters to be measured with fluorescent videomicroscopy as previously described. After a 60-minute equilibration period, the control relation between vascular diameter and perfusion pressure was determined at pressures of 80, 120, and 160 mm Hg. After the pressure was changed, an equilibration period of 5 minutes was allowed before the new diameter measurements were taken.

After the control pressure–diameter relations were determined, the effects of addition of N^ω-nitro-L-arginine (L-NOARG, 100 μM) to the perfusate were studied. Then, L-arginine (1 mM), the precursor of NO production, was added to the perfusate to determine if the effects of L-NOARG could be reversed. The perfusate and bath then were changed to a calcium-free solution, and the pressure–diameter relations were re-
Imig and Roman  Nitric Oxide Modulates Renal Vascular Tone  771

Control

M-ABQ - A K-lfiG  + L-1HG - A CsFres

600

400

I

V

I

s

400

120 ISO

UB

I

BO 120 160

Perfusion Pressure (mmHg)

FIGURE 1. Line plots show effects of N*-nitro-L-arginine (L-NOARG) and L-arginine on pressure–diameter relation of arcuate (n=11 vessels, six kidneys) and interlobular (n=9) arteries and proximal (n=14) and distal (n=11) portions of afferent arteriole perfused with physiological salt solution (PSS), PSS containing L-NOARG, PSS containing L-NOARG and L-arginine and calcium-free solution. N-ARG, N*-nitro-L-arginine; L-ARG, L-arginine. *Significant difference from diameter measured at 80 mm Hg with same perfusate; †significant difference from corresponding value measured in PSS-perfused preparation.

determined to determine the degree of active vascular tone in the preparation.

Vascular Pressure Measurements

Pressures were measured with a servonull micropipette pressure system (model 5A, Instrumentation for Physiology & Medicine, Inc., San Diego, Calif.) and siliconized glass micropipettes (1–3 μm o.d.). In these experiments, the preparation was illuminated with a fiber-optic light and viewed through a binocular microscope (DCR Stereomicroscope, Carl Zeiss, Oberkochen, FRG). In each experiment, glomerular capillary pressure was measured at perfusion pressures of 80, 120, and 160 mm Hg. Then, paired pressure measurements were obtained at upstream vascular sites (distal afferent arteriole, proximal afferent arteriole, interlobular and main arcuate arteries) at each level of perfusion pressure. Experiments were performed in preparations perfused with PSS (n=4), PSS plus L-NOARG (n=4), and calcium-free PSS (n=4).

Glomerular capillary pressure autoregulatory indexes were calculated using the following formula:

\[ \text{GCP AI} = \left( \frac{(\text{GCP}_2 - \text{GCP}_1) + \text{GCP}_1}{(\text{RPP}_2 - \text{RPP}_1) + \text{RPP}_1} \right) \]

where GCP AI is glomerular capillary pressure autoregulatory indexes, RPP, is renal perfusion pressure of 80 mm Hg, and RPP, are the radii of the vessels measured in PSS or PSS plus L-NOARG and in calcium-free PSS, respectively. Data are presented as mean±SEM. Significance of differences in mean values between groups was evaluated with two-way analysis of variance for repeated measures followed by Duncan’s multiple range test. A value of p<0.05 was considered statistically significant.

Results

Figure 1 summarizes the effects of blockade of NO production with L-NOARG on the pressure–diameter relations of the preglomerular vasculature of deep nephrons. Under control conditions, the diameters of the main arcuate and interlobular arteries increased by 14% and 6%, respectively, when perfusion pressure was increased from 80 to 160 mm Hg. In contrast, the diameters of the proximal and distal afferent arterioles decreased by 3% and 7%, respectively, in response to the same stimulus. Addition of L-NOARG to the perfusate had no effect on the basal diameters of the arcuate and interlobular arteries (measured at 80 mm Hg). However, the diameter of the interlobular arteries decreased by 2% rather than increased in the presence of L-NOARG when perfusion pressure was elevated from 80 to 160 mm Hg. L-NOARG reduced the basal diameters of afferent arterioles by 7%, but it did not alter their response to an elevation in perfusion pressure. Addition of L-arginine to the perfusate completely reversed the effects of L-NOARG. Removal of calcium from the perfusate and bath increased the diameters of all vascular segments at all perfusion pressures studied.

Table 1 presents the pressure distribution within the preglomerular vasculature. Control glomerular capillary pressure in preparations perfused with PSS averaged 43
TABLE 1. Pressure Distribution in Juxtamedullary Nephron Microvascular Preparation

<table>
<thead>
<tr>
<th></th>
<th>Perfusion pressure (mm Hg)</th>
<th>Physiological salt solution</th>
<th>N^*^-Nitro-L-arginine</th>
<th>Ca(^{2+})-Free solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80</td>
<td>120</td>
<td>160</td>
<td>80</td>
</tr>
<tr>
<td>Arcuate artery</td>
<td>79±1</td>
<td>118±1</td>
<td>154±2</td>
<td>76±1</td>
</tr>
<tr>
<td>Interlobular artery</td>
<td>74±1</td>
<td>108±5</td>
<td>139±5</td>
<td>74±2</td>
</tr>
<tr>
<td>Proximal afferent arteriole</td>
<td>68±1</td>
<td>91±3</td>
<td>102±6</td>
<td>66±2</td>
</tr>
<tr>
<td>Distal afferent arteriole</td>
<td>50±2</td>
<td>64±5</td>
<td>73±5</td>
<td>53±3</td>
</tr>
<tr>
<td>Glomerulus</td>
<td>43±1</td>
<td>48±1</td>
<td>52±2</td>
<td>45±1</td>
</tr>
</tbody>
</table>

Values are mean±SEM obtained from four to seven vessels from at least four preparations.

'Significantly different from physiological salt solution (PSS) and PSS plus N^*^-nitro-L-arginine at same perfusion pressure; tsignificantly different from PSS at same perfusion pressure.

Pressures were elevated in the afferent arterioles and glomerulus at all perfusion pressures studied compared with corresponding values in PSS- and L-NOARG-perfused vasculatures when calcium was removed from the perfusate and bath. Under these conditions, glomerular capillary pressure was poorly autoregulated (GCP AI, 0.53±0.03), as it increased by 30 mm Hg when perfusion pressure was elevated from 80 to 160 mm Hg. Although poorly autoregulated, the calcium-free GCP AI indicates that there is significant autoregulation due to the inherent structural arrangement of the juxtamedullary vasculature.

Measurement of pressures and diameters at each vascular site allow for determination of active wall tension (Figure 2). There was significant basal active tone in all segments of the renal vasculature at a control perfusion pressure of 80 mm Hg (Table 1). However, autoregulation of glomerular capillary pressure was significantly enhanced, and GCP AI decreased to 0.02±0.04. Glomerular capillary pressure increased by only 1 mm Hg when perfusion pressure was raised from 80 to 160 mm Hg in these kidneys. This was due to an increase of 8 mm Hg in the pressure drop along the proximal portion of the afferent arteriole, which resulted in a significantly lower pressure presented to the distal afferent arteriole in comparison with PSS-perfused preparation.
increase active wall tension when perfusion pressure was increased to 160 mm Hg. In afferent arterioles, L-NOARG significantly enhanced basal active wall tension, but it did not alter the response to elevations in perfusion pressure.

**Discussion**

Recent studies have demonstrated that NO is tonically released and influences renal hemodynamics. Acute blockade of NO synthesis in rats reduces renal blood flow and glomerular filtration rate. The present study examined potential sites of action of NO in the renal circulation using the juxtamedullary microvascular preparation perfused in vitro with a cell-free perfusate.

In the present study, addition of L-NOARG to the perfusate reduced the diameter of the afferent arterioles at all perfusion pressures studied, whereas the diameter of the arcuate and interlobular arteries was not significantly altered. L-Arginine completely reversed the effects of L-NOARG. These findings are consistent with a recent report that the diameter of in vitro perfused rabbit afferent arterioles is reduced by inhibitors of NO production.

To examine the functional significance of these changes in afferent arteriolar diameter, we studied the effects of L-NOARG on the pressure distribution within the juxtamedullary vasculature and on autoregulation of glomerular capillary pressure. Under control conditions (PSS at 80 mm Hg), most of the preglomerular pressure drop occurred along the afferent arteriole. Glomerular capillary pressure was well autoregulated and increased by only 10 mm Hg in response to an elevation in perfusion pressure from 80 to 160 mm Hg. Inhibition of NO synthesis significantly enhanced autoregulation of glomerular capillary pressure. Glomerular capillary pressure increased by only 1 mm Hg when perfusion pressure was increased over this same range. The potentiation of the autoregulatory response by L-NOARG appears to be due to enhanced active vascular tone and reduced diameter of the afferent arteriole, which increased the pressure drop along its length.

Recent results have demonstrated that endothelium-derived relaxing factor modulates the vasoconstrictor effects of angiotensin II and endothelin on the afferent arteriole. Romero et al have also suggested that NO release may be stimulated at elevations in perfusion pressure and may oppose renal autoregulation. The present results indicating that autoregulation of glomerular capillary pressure in the in vitro perfused juxtamedullary microvascular preparation was improved by L-NOARG support this view.

The extent to which the present in vitro findings can be applied to the in vivo situation and the suitability of the isolated juxtamedullary preparation as a model for renal autoregulation in vivo remains to be determined. In a recent mathematical modeling study, we reported that the degree of constriction of the afferent arteriole in response to elevations in perfusion pressure is about half of that needed to explain the observed degree of glomerular capillary pressure autoregulation and that some other mechanism may be involved. One possibility is that postglomerular resistance may decrease slightly with elevations in perfusion pressure so that glomerular capillary pressure but not flow is autoregulated in this preparation. Another possibility is that the network basis of the renal circulation provides some degree of structural autoregulation such that elevations in perfusion pressure redistribute flow from areas of the preparation exhibiting autoregulation toward other regions not under study. The present findings indicating substantial autoregulation of glomerular capillary pressure in this preparation after removal of calcium from the perfusate and bath are consistent with this interpretation.

To further examine whether shear-related changes in NO release may oppose autoregulatory responses, the effects of L-NOARG on active wall tension at various sites in the renal vasculature were determined as a function of perfusion pressure. Even though arcuate and interlobular arteries did not constrict after an elevation in perfusion pressure, all segments of the retrovascular arteriole responded with an increase in active wall tension. L-NOARG had little effect on active tone in the main arcuate arteries, but it did augment active wall tension at elevated perfusion pressures in interlobular arteries. The major effect of L-NOARG, however, was to increase active vascular tone in the afferent arteriole without altering its response to changes in perfusion pressure.

These results suggest that NO is tonically released and is a determinant of vascular tone in afferent arterioles under the present experimental conditions. The enhanced autoregulation of glomerular capillary pressure after inhibition of NO synthesis was likely due to a decrease in the diameters and increase in pressure drop along the length of the afferent arteriole rather than to a potentiation of their myogenic or tubuloglomerular feedback response to changes in perfusion pressure.

In summary, these results suggest that NO alters primarily vascular tone in the afferent arterioles in the juxtamedullary microvasculature of the rat perfused in vitro with a cell-free media and thereby modifies the ability of the preglomerular vasculature to autoregulate glomerular capillary pressure.

**References**


Nitric oxide modulates vascular tone in preglomerular arterioles.
J D Imig and R J Roman

Hypertension. 1992;19:770-774
doi: 10.1161/01.HYP.19.6.770

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on
the World Wide Web at:
http://hyper.ahajournals.org/content/19/6_Pt_2/770