Evidence for the Role of Nitric Oxide in Macula Densa Control of Glomerular Hemodynamics

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Abstract

There is evidence that nitric oxide, an endothelium-derived relaxing factor, may be produced by the macula densa, as well as by blood vessels, within the kidney. To examine the role of nitric oxide in macula densa control of glomerular hemodynamics directly, we performed in vitro microperfusions of both rabbit afferent arterioles (with the glomerulus intact) and adherent tubular segments consisting of portions of the thick ascending limb, macula densa, and early distal tubule. While keeping afferent arteriolar pressure constant at 60 mmHg, we examined the effect of N^w-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, added to a macula densa perfusate. When the macula densa perfusate was changed from low to high NaCl, the diameter of the arterioles decreased from 16.3 ± 1.0 to 14.0 ± 1.1 μm ($n = 10$; $P < 0.001$). Addition of 10^{-5} M L-NAME to the high NaCl solution further decreased the diameter to 11.9 ± 1.1 μm ($P < 0.001$). In contrast, when macula densa perfusion was maintained with the low NaCl solution, addition of L-NAME had no effect. L-NAME–induced constriction was completely reversed by adding 10^{-3} M L-arginine (the precursor of nitric oxide) but not D-arginine (an inactive isomer) to the macula densa perfusate. We confirmed that perfusing the macula densa with L-NAME did not affect the vasodilator action of acetylcholine added to the lumen of the afferent arteriole, indicating that NO synthesis by the arteriole was not altered. Thus, our findings suggest that the macula densa may produce nitric oxide, which in turn modulates the afferent arteriolar constriction induced by high concentrations of NaCl at the macula densa. (*J. Clin. Invest.* 1993. 92:1093–1098.) Key words: endothelium-derived relaxing factor • microperfusion • afferent arteriole • tubuloglomerular feedback

Introduction

In each nephron of the mammalian kidney, the tubule returns to the hilus of the parent glomerulus, forming the juxtaglomerular apparatus that displays a unique arrangement of glomerular afferent arteriole (Af-Art)[1] and efferent arteriole (Ef-Art), interstitial cells and macula densa (MD) (1). Because of this intimate anatomical relationship, it has long been suggested that the MD may somehow sense changes in the composition of the tubular fluid and control the GFR (2) by a mechanism called tubuloglomerular feedback. It has been shown that tubuloglomerular feedback plays an important role in renal autoregulation, as well as homeostasis of fluid volume and electrolytes (3–6). Alteration of tubuloglomerular feedback has been reported in many physiological and pathological conditions, such as hypertension, high protein intake, uninephrectomy, hyperglycemia, and dehydration (7, 8). Numerous in vivo single-nephron micropuncture studies have established that increased NaCl concentration and/or osmolality of the tubular fluid at the MD decreases single-nephron GFR. Most studies indicate that changes in Af-Art resistance play a major (and most likely a dominant) role in altering GFR (9). Despite intensive investigation, however, the mechanism of MD-mediated glomerular hemodynamics remains incompletely understood.

Since the discovery of endothelium-derived relaxing factor (EDRF) by Furchgott and Zawadzki (10), it has been studied extensively in various organs including the kidney. An EDRF has been identified as nitric oxide (NO), which is synthesized enzymatically from the amino acid L-arginine (11). Although studies have demonstrated that NO plays an important role in the control of renal function (12), its site and mechanism of action are yet to be elucidated. We have previously shown that in isolated microperfused rabbit Af-Arts, inhibition of NO synthesis not only decreases basal diameter but also augments the vasoconstrictor action of angiotensin II (13). This suggests that NO produced locally within the Af-Art is an important determinant of glomerular hemodynamics. On the other hand, NO may directly influence tubular transport independently of renal hemodynamics (14). Furthermore, a recent study suggests that type I NO synthase, which is a different isoform from the type III found in the endothelium, is abundant in the MD cells (15).

In this study, we tested the hypothesis that the MD cells produce NO, which in turn modulates the Af-Art constriction induced by increased NaCl concentration at the MD. For this, we used a novel and recently established in vitro preparation in which both the isolated Af-Art and attached MD are microper-

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1. *Abbreviations used in this paper:* Af-Art, afferent arteriole; EDRF, endothelium-derived relaxing factor; Ef-Art, efferent arteriole; L-NAME, N^w-nitro-L-arginine methyl ester; MD, macula densa; NO, nitric oxide.
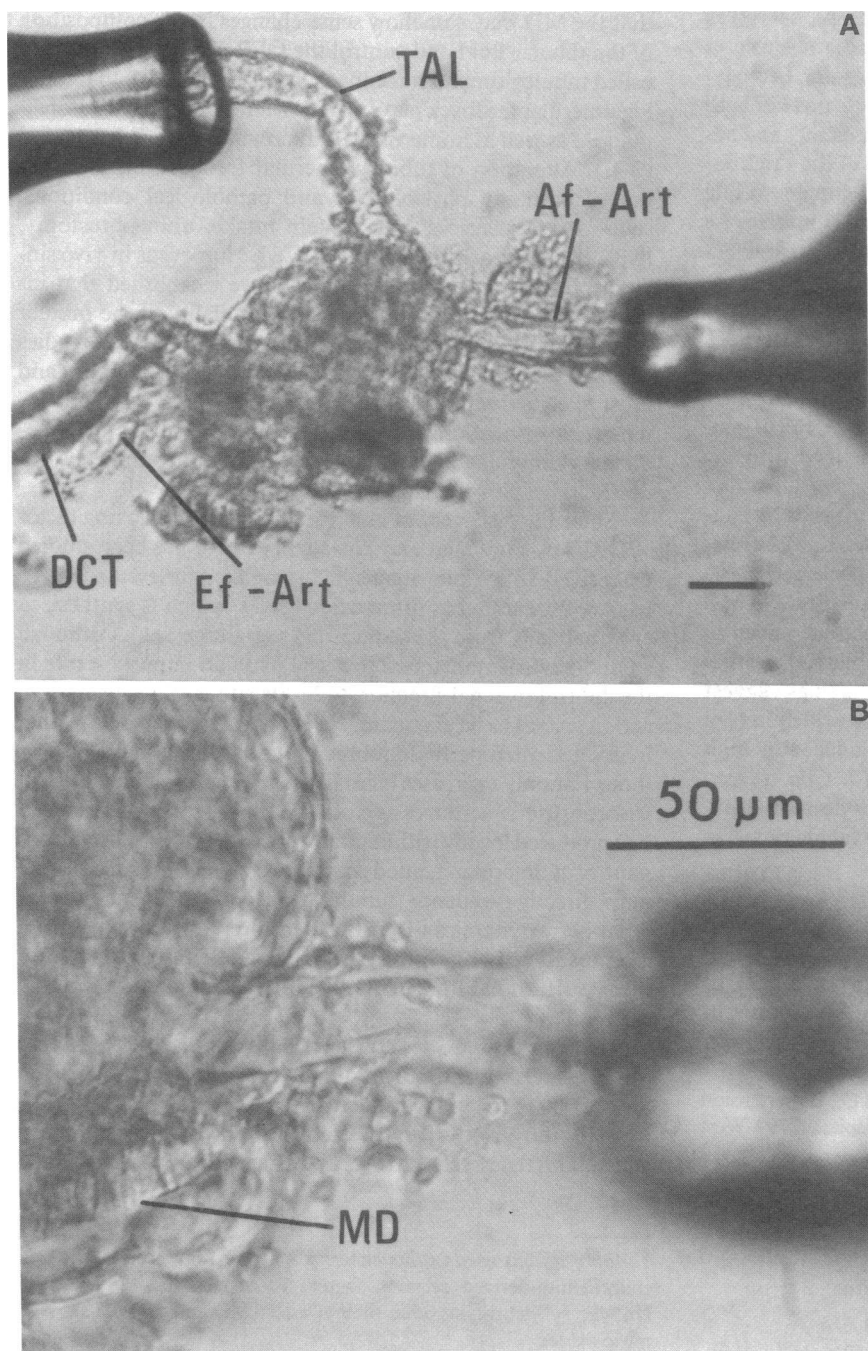
fused simultaneously (Fig. 1). We have previously shown that increasing NaCl concentration of the MD perfusate causes constriction of the Af-Art, particularly in the distal segment (16, 17). Our preparation has the advantage of allowing us to observe the Af-Art directly in the absence of systemic hemodynamic and hormonal influences while controlling both pressure in the Af-Art and the composition of the tubular fluid at the MD.

Methods

Isolation and microperfusion of the rabbit Af-Art with MD attached

We used a method similar to that described previously to isolate and microperfuse Af-Arts with MD attached (13, 16). Briefly, young male

New Zealand white rabbits (1.5–2.0 kg) fed standard rabbit chow (Ralston Purina, St. Louis, MO), and given tap water ad libitum were anesthetized intravenously with sodium pentobarbital (40 mg/kg) and given an i.v. injection of heparin (500 U). The kidneys were removed and sliced along the corticomedullary axis. Slices were placed in ice-cold MEM (Gibco, Grand Island, NY) containing 5% BSA (Sigma Chemical Co., St. Louis, MO) and dissected under a stereomicroscope (model SZH; Olympus, Tokyo, Japan) as described previously. From each rabbit, a single superficial Af-Art and its intact glomerulus were microdissected together with adherent tubular segments consisting of portions of the thick ascending limb of Henle, MD, and early distal tubule (Fig. 1). Using a micropipette, the microdissected complex was transferred to a temperature-regulated chamber mounted on an inverted microscope (model IMT-2; Olympus) with Hoffman modulation. Both the Af-Art and the end of either the distal tubule or thick ascending limb were cannulated with an array of glass pipettes as de-

Figure 1. Simultaneous perfusion of a glomerular afferent arteriole and attached macula densa. (*A*) *DCT*, distal convoluted tubule; *TAL*, thick ascending limb of Henle's loop. Bar, 50 μm. (*B*) After perfusion has been established, both the MD and distal afferent arteriole can be visualized. Bar, 50 μm.

scribed previously (13, 16). Intraluminal pressure was measured by Landis' technique, using a fine pipette introduced into the Af-Art through the perfusion pipette. The Af-Art was perfused with oxygenated medium 199 (95% O_2 and 5% CO_2) containing 5% BSA, and intraluminal pressure was maintained at 60 mmHg throughout the experiment. The MD was perfused with a modified Krebs-Ringer bicarbonate buffer (oxygenated to pH 7.4) at a rate of 10 nl/min.

The bath consisted of 100 μl medium 199 containing 0.1% BSA and was exchanged continuously at a rate of 1 ml/min. Microdissection and cannulation were completed within 90 min at 8°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. Once the temperature was stable, a 30-min equilibration period was allowed before taking any measurements. Images were displayed at magnifications up to 1,980 and recorded with a video system consisting of a camera (model DXC-755; Sony, Tokyo, Japan), monitor (model PVM1942Q; Sony), and video recorder (model EDV-9500; Sony). The diameter of the distal Af-Art was measured with an image-analysis system (Fryer, Carpentersville, IL).

Experimental protocols

Response to N^w-nitro-L-arginine methyl ester (L-NAME) added to MD perfusate containing either high or low NaCl. In four experiments, the MD was perfused with a modified Krebs-Ringer bicarbonate buffer containing high NaCl from the equilibration period to the end of the experiment. The high NaCl solution had the following composition: 115 mM NaCl, 15 mM $NaHCO_3$, 10 mM sodium acetate, 0.96 mM NaH_2PO_4, 0.24 mM Na_2HPO_4, 5 mM KCl, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, and 5.5 mM glucose (total osmolality, 280 mosmol/kg). After the 30-min equilibration period, two increasing concentrations of L-NAME (Sigma) (10^{-5} and 10^{-4} M) were added to the MD perfusate. Luminal diameters of the Af-Arts were measured immediately before adding L-NAME and observed for 20 min at each dose. Because of time constraints, we tested only two concentrations of L-NAME for each preparation, since our previous studies have shown that MD-mediated Af-Art responses are consistent over a 45-min period (16, 17).

Since we observed that Af-Art responses reached a maximum at 10^{-5} M (see the Results), we used lower concentrations (10^{-6} and 10^{-5} M) of L-NAME in subsequent studies. In seven experiments, we examined the effect of L-NAME when the NaCl concentration at the MD was low. The MD was perfused with a low NaCl solution that was identical to the high NaCl solution, except that NaCl was removed without correcting osmolality (88 mosmol/kg). After the equilibration period, L-NAME was added to the MD perfusate for 20 min at each dose (10^{-5} and 10^{-4} M). In another 10 experiments, we examined the influence of the high-NaCl MD perfusate on basal diameter, as well as changes induced by the addition of L-NAME. The MD was perfused with the low NaCl solution during the equilibration period, after which the perfusate was changed to the high NaCl solution for the remainder of the experiment. 5 min later, L-NAME was added to the MD perfusate and Af-Arts observed for 20 min at each dose.

Effect of L- or D-arginine on L-NAME–induced constriction. We examined whether Af-Art vasoconstriction induced by adding L-NAME to a high NaCl MD perfusate is specifically caused by inhibition of NO synthesis from L-arginine (11). While perfusing the MD with the high NaCl solution, we first added 10^{-5} M L-NAME, after which either L-arginine or its inactive isomer, D-arginine, at 10^{-3} M (Sigma) was added to the perfusate together with L-NAME.

Effect of intraarteriolar acetylcholine during L-NAME perfusion of the MD. We conducted experiments to rule out the possibility that the observed changes in Af-Art diameter were secondary to inhibition of NO synthesis in the Af-Art rather than the MD cells. While perfusing the MD with 10^{-5} M L-NAME, we constricted the Af-Art to 60–70% of basal diameter by adding 10^{-7} M norepinephrine to the bath, after which acetylcholine (10^{-6} and 10^{-5} M) was added only to the lumen of the Af-Arts ($n = 6$).

Statistics

Values were expressed as mean±SEM. A paired t test was used to examine whether the diameter at a given concentration was different from the control value. Analysis of covariance was used to examine whether the change in diameter at a given concentration was different between groups. For both analyses, $P < 0.025$ (0.05/2; Bonferroni adjustment) was considered significant.

Results

Response to L-NAME added to MD perfusate containing either high or low NaCl. Figure 2 shows an example of the response to 10^{-5} and 10^{-4} M L-NAME added to the MD perfusate that had a high NaCl concentration (Na^+, 144 meq/liter; and Cl^-, 122 meq/liter). It is evident that L-NAME at both concentrations caused substantial constriction of the Af-Art. Of note is the fact that 10^{-5} and 10^{-4} M L-NAME caused similar constriction, suggesting that the response had already reached a maximum at 10^{-5} M. In four such experiments, the decrease in diameter was 18.4±6.5 and 20.2±6.9% at 10^{-5} and 10^{-4} M, respectively.
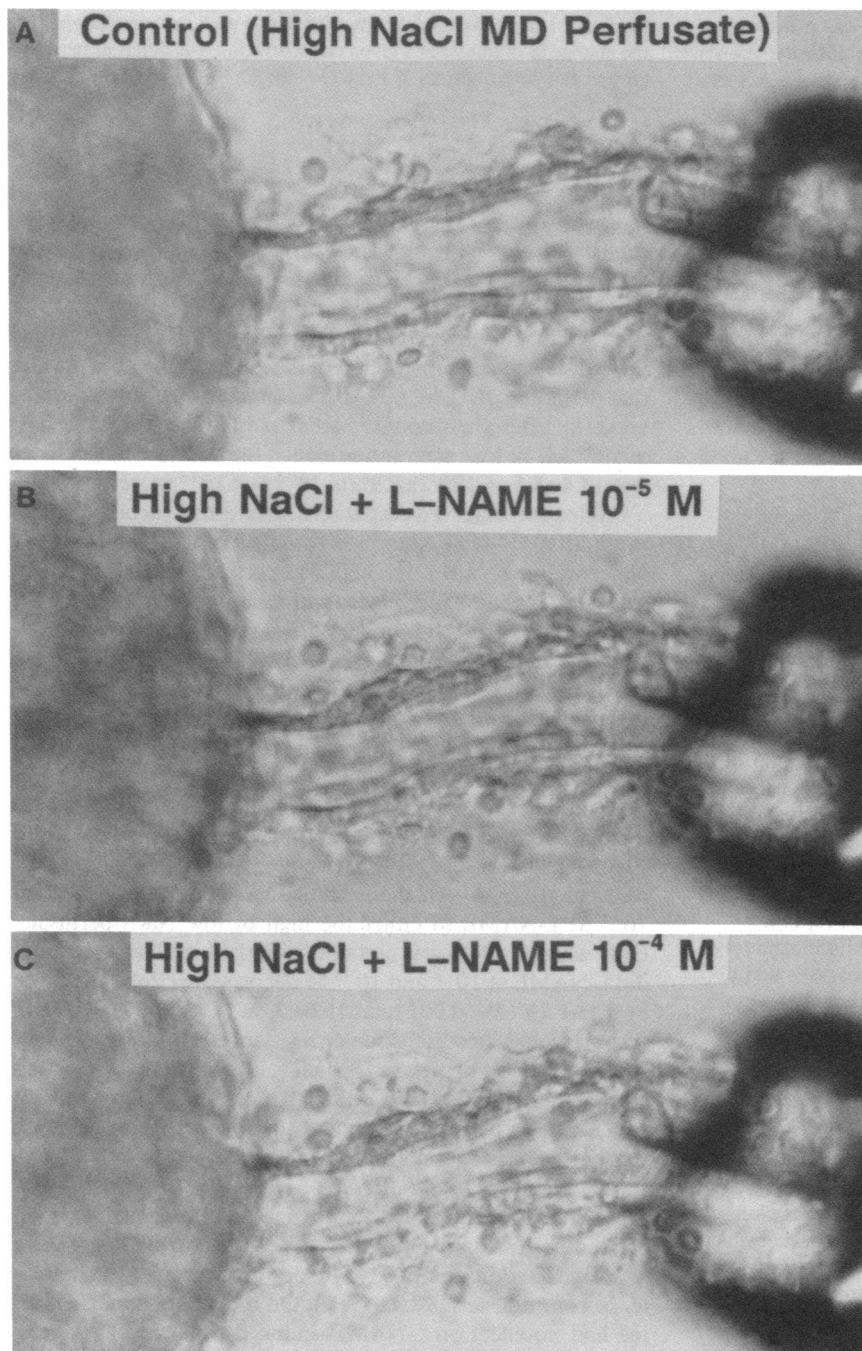
Since the observed response had already reached a maximum at 10^{-5} M, we examined the effect of L-NAME at lower concentrations. When the MD was perfused with a low NaCl solution (Na^+, 26 meq/liter; and Cl^-, 7 meq/liter), Af-Art diameter was 17.1±1.3 μm ($n = 7$) at basal and remained unchanged after addition of L-NAME (17.1±1.7 and 17.2±1.5 μm at 10^{-6} and 10^{-5} M, respectively). When the MD perfusate was changed from low to high NaCl, basal diameter decreased from 16.3±1.0 to 14.0±1.1 μm ($n = 10$; $P < 0.001$). In contrast to L-NAME's lack of effect with the low NaCl perfusate, addition of L-NAME (10^{-6} and 10^{-5} M) to the high NaCl solution further decreased the diameter to 12.8±1.0 ($P < 0.01$) and 11.9±1.1 μm ($P < 0.001$), respectively. Fig. 3 depicts the percent change in Af-Art diameter induced by adding 10^{-6} and 10^{-5} M L-NAME to either the high or low NaCl perfusate. Only when the MD was perfused with the high NaCl solution, did L-NAME at 10^{-6} and 10^{-5} M decrease arteriolar diameter by 8.1±1.7% ($P < 0.001$) and 15.6±2.8% ($P < 0.001$), which in theory, would increase vascular resistance by 40 and 90%, respectively. Fig. 4 depicts individual values for changes in arteriolar diameter induced by adding 10^{-5} M L-NAME to the high NaCl MD perfusate. It is evident that the arteriolar response was both consistent and reproducible.

Effect of L- or D-arginine on L-NAME–induced constriction. L-NAME (10^{-5} M) added to the high NaCl MD perfusate decreased Af-Art diameter to 84.5±1.9% of control ($P < 0.01$; $n = 5$), while addition of 10^{-3} M L-arginine reversed the diameter to control levels (98.9±4.1%). On the other hand, D-arginine had no effect on L-NAME–induced constriction: with L-NAME, the diameter decreased to 76.2±2.6% of control ($P < 0.01$; $n = 4$), and remained unchanged after addition of 10^{-3} M D-arginine (74.4±5.3%).

Effect of intraarteriolar acetylcholine during L-NAME perfusion of the MD. While the MD was perfused with 10^{-5} M L-NAME, 10^{-7} M norepinephrine was added to the bath; it constricted the Af-Art to 62.0±5.5% of basal diameter ($P < 0.0001$; $n = 8$). When acetylcholine was added only to the lumen of the Af-Art at 10^{-6} and 10^{-5} M, the diameter increased to 101±11.9 ($P < 0.003$ vs norepinephrine) and 110±11.9% of baseline ($P < 0.002$), respectively.

Discussion

Mundel et al. (15) have recently shown that immunoreactivity, enzymatic activity as well as expression of mRNA of type I NO
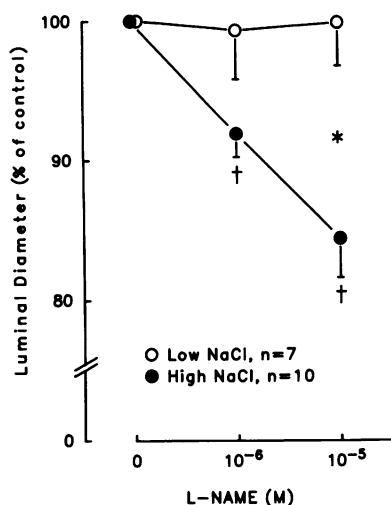
A Control (High NaCl MD Perfusate)

B High NaCl + L-NAME 10^{-5} M

C High NaCl + L-NAME 10^{-4} M

Figure 2. Afferent arteriolar constriction induced by 10^{-5} and 10^{-4} M L-NAME (an inhibitor of nitric oxide synthesis) added to a macula densa perfusate with a high NaCl concentration. Note that 10^{-5} and 10^{-4} M L-NAME caused similar constriction of the afferent arteriole, which is strongest in the distal segment.

synthase are more abundant in the cytoplasm of MD cells than in any other structure (including the vascular endothelium) within the kidney. Their study provides a morphological basis for the role of NO in the control of glomerular hemodynamics and/or renin release by the MD. We now present direct evidence that the MD may indeed produce NO, which, in turn, modulates the vascular response of the Af-Art, a major vascular segment that controls glomerular hemodynamics.

The present study demonstrates that adding L-NAME (an inhibitor of NO synthesis) only to the MD perfusate constricts the Af-Art. Constriction is seen with a high but not a low NaCl MD perfusate, suggesting that NO produced within the MD

cells and/or juxtaglomerular interstitium modulates the Af-Art constriction induced by high concentrations of NaCl at the MD. Since L-NAME-induced vasoconstriction is abolished by L-arginine (the precursor of NO), but not D-arginine (an inactive isomer of L-arginine), the constriction observed with intratubular L-NAME seems to be specifically caused by inhibition of NO synthesis from L-arginine. Our results are consistent with a recent in vivo micropuncture study by Wilcox et al. (18, 19), who reported that perfusing the loop of Henle with L-NAME lowered the stop-flow pressure, and that this decrease was abolished by adding L-arginine.
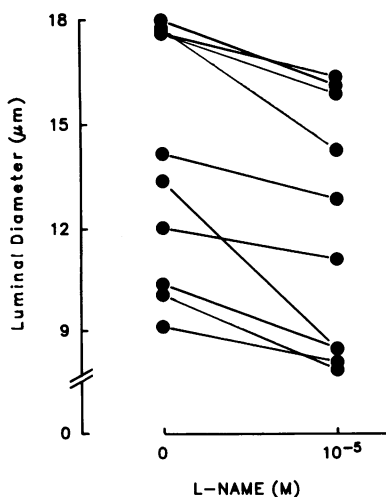
The MD perfusate issues into bath in our preparation. How-

Figure 3. Changes in afferent arteriolar diameter induced by L-NAME (an inhibitor of nitric oxide synthesis) added to the macula densa perfusate. The macula densa was perfused with a modified Krebs-Ringer bicarbonate buffer containing either high NaCl (●) or low NaCl (○) throughout the experiment. After taking control measurements, L-NAME (10^{-6} and 10^{-5} M) was added to the macula densa perfusate and the arteriole observed for 20 min. $\dagger P < 0.001$ compared with control, $*P < 0.003$ for low vs high NaCl.

ever, it is unlikely that the L-NAME concentration in the bath reached high enough concentrations to affect the Af-Art, since the rate of MD perfusion was 10 nl/min, while the bath was 100 μl and was exchanged continuously at a rate of 1 ml/min. Nevertheless, we conducted experiments to rule out the possibility that the observed changes in Af-Art diameter were secondary to inhibition of NO synthesis in the Af-Art rather than in the MD cells. While perfusing the MD with 10^{-5} M L-NAME, we examined the vasodilator action of acetylcholine (which stimulates NO synthesis by the endothelium) added directly to the lumen of Af-Arts, which had been preconstricted to 65% of control diameter with norepinephrine. Acetylcholine at both 10^{-6} and 10^{-5} M caused significant vasodilation, completely reversing the diameter to 101 and 110% of control values, respectively. The acetylcholine-induced dilation was similar to that observed in nontreated Af-Arts, suggesting that NO synthesis in the Af-Art was not significantly altered under our experimental conditions. Furthermore, when L-NAME was added to the lumen of the Af-Arts at doses of 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M, the diameter decreased in a dose-dependent manner by 2.9±3.3, 5.3±2.2, 14.1±4.3 and 21.8±3.0%, respec-

Figure 4. Individual values for changes in arteriolar diameter induced by 10^{-5} M L-NAME added to a high NaCl macula densa perfusate ($n = 10$).

tively ($n = 11$). Thus, even though the Af-Art endothelium was exposed to L-NAME directly, the maximum arteriolar response was not obtained until the concentration reached 10^{-4} M. On the other hand, when L-NAME was added to the MD perfusate, it induced a maximum response already at doses as low as 10^{-5} M (Fig. 2). The disparate dose-response curves suggest that the mechanism of Af-Art contraction induced by these two routes of L-NAME administration is different.

The mechanism by which L-NAME induced Af-Art constriction with a high- but not a low-NaCl MD perfusate is not clear. It may be that in response to increased tubular transport, the MD produced not only a vasoconstrictor signal (as yet undefined) but also NO, which opposed each other's actions at the level of Af-Art. Alternatively, the MD may have continuously released NO independently of tubular transport; however, when the MD was perfused with the low NaCl solution, the vasodilator action of NO may have not been apparent because of already low basal Af-Art resistance, whereas with the high NaCl perfusate NO may have efficiently counteracted the vasoconstrictor signal. Thus inhibition of NO with L-NAME would result in significant Af-Art constriction only when the MD was perfused with a high NaCl solution. In addition, it may be possible that NO altered NaCl transport at the MD, as a direct tubular actions of NO was reported in a mouse cortical collecting duct cell line. Thus NO within the MD (regardless of how it was formed) may have inhibited its own tubular transport, thereby limiting the rate of synthesis of the vasoconstrictor signal induced by high NaCl concentrations at the MD. Further experiments are necessary to clarify these possibilities.

We have previously presented evidence that the Af-Art produces NO, which is important in the control of basal tone, as well as the vascular response to both angiotensin II and endothelin (13, 20). The present study provides evidence that NO within the MD and/or juxtaglomerular interstitium may function independently of that produced by the renal vasculature. It is conceivable that the activity of NO in these two compartments is differentially regulated under various physiological and pathological conditions. Interestingly, a recent preliminary report indicates that immunoreactivity of NO synthase in the MD was greatly reduced by dietary sodium restriction, suggesting that decreased activity of NO in the MD may contribute to augmented TGF response seen during sodium restriction (18). In addition to glomerular hemodynamics, the MD controls renin release in response to changes in the composition of tubular fluid (6). Therefore, it may be possible that NO produced in the MD versus Af-Art endothelium may have different effects on renin release.

In conclusion, we have demonstrated that perfusion of the MD with L-NAME causes constriction of the Af-Art, which seems to be caused by inhibition of NO synthesis (from L-arginine) in the MD and/or juxtaglomerular interstitium but not in the Af-Art. L-NAME-induced constriction is seen only when the MD is perfused with high but not low NaCl, suggesting that MD cells may produce NO, which in turn modulates the Af-Art constriction induced by increased NaCl concentration at the MD. Thus, our data provide direct evidence for the functional role of NO in the juxtaglomerular apparatus.

Acknowledgments

References

1. Barajas, L., and K. Powers. 1984. The structure of the juxtaglomerular apparatus (JGA) and the control of renin secretion: an update. *J. Hypertens.* 2(Suppl. 1):3-12

2. Goormaghtigh, N. 1945. Facts in favor of an endocrine function of the renal arterioles. *J. Pathol.* 57:392-393

3. Bell, P. D., M. Franco, and L. G. Navar. 1987. Calcium as a mediator of tubuloglomerular feedback. *Annu. Rev. Physiol.* 49:275-293

4. Briggs, J. P., and J. Schnermann. 1987. The tubuloglomerular feedback mechanism: functional and biochemical aspects. *Annu. Rev. Physiol.* 49:251-273

5. Navar, L. G., D. W. Ploth, and P. D. Bell. 1980. Distal tubular feedback control of renal hemodynamics and autoregulation. *Annu. Rev. Physiol.* 42:557-571

6. Schnermann, J., and J. P. Briggs. 1992. Function of the juxtaglomerular apparatus: control of glomerular hemodynamics and renin secretion. *In* The Kidney: Physiology and Pathophysiology. D. W. Seldin and G. Giebisch, editors. Raven Press, Ltd. New York. 1249-1290.

7. Arendshorst, W. J. 1987. Altered reactivity of tubuloglomerular feedback. *Annu. Rev. Physiol.* 49:295-317

8. Wright, F. S., and M. D. Okusa. 1990. Functional role of tubuloglomerular feedback control of glomerular filtration. *Adv. Nephrol.* 19:119-134

9. Briggs, J. P., and F. S. Wright. 1979. Feedback control of glomerular filtration rate: site of the effector mechanism. *Am. J. Physiol.* 236:F40-F47

10. Furchgott, R. F., and J. V. Zawadzki. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond.).* 288:373-376

11. Moncada, S., R. M. J. Palmer, and E. A. Higgs. 1992. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109-142

12. Romero, J. C., V. Lahera, M. G. Salom, and M. L. Biondi. 1992. Role of the endothelium-dependent relaxing factor nitric oxide on renal function. *J. Am. Soc. Nephrol.* 2:1371-1387

13. Ito, S., C. S. Johnson, and O. A. Carretero. 1991. Modulation of angiotensin II-induced vasoconstriction by endothelium-derived relaxing factor in the isolated microperfused rabbit afferent arteriole. *J. Clin. Invest.* 87:1656-1663

14. Stoos, B., O. A. Carretero, R. D. Farhy, G. Scicli, and J. L. Garvin. 1992. Endothelium-derived relaxing factor inhibits transport and increases cGMP content in cultured mouse cortical collecting duct cells. *Clin. Invest.* 89:761-765

15. Mundel, P., S. Bachmann, M. Bader, A. Fischer, W. Kummer, B. Mayer, and W. Kritz. 1992. Expression of nitric oxide synthase in kidney macula densa cells. *Kidney Int.* 42:1017-1019

16. Ito, S., and O. A. Carretero. 1990. An in vitro approach to the study of macula densa-mediated glomerular hemodynamics. *Kidney Int.* 38:1206-1210

17. Ito, S., and O. A. Carretero. 1991. Macula densa control of glomerular hemodynamics. *Kidney Int.* 39(Suppl. 32):S83-S85

18. Wilcox, C. S., W. J. Welch, A. Tojo, C. C. Tisher, and H. H. W. Schmidt. 1992. A constitutive nitric oxide synthase localizes to macula densa and mediates a salt-sensitive vasodilator component of tubuloglomerular feedback. *J. Am. Soc. Nephrol.* 3:556. (Abstr.).

19. Wilcox, C. S., W. J. Welch, F. Murad, S. S. Grosse, G. Taylor, R. Levi, and H. H. W. Schmidt. 1992. Nitric oxide synthase in macula densa regulates glomerular capillary pressure. *Proc. Natl. Acad. Sci. USA.* 89:11993-11997.

20. Ito, S., L. A. Juncos, N. Nushiro, C. S. Johnson, and O. A. Carretero. 1991. Endothelium-derived relaxing factor modulates endothelin action in afferent arterioles. *Hypertension (Dallas).* 17:1052-1056