Mechanism underlying diuretic effect of L-NAME at a subpressor dose

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Liang, Mingyu, Theresa J. Berndt, and Franklyn G. Knox. Mechanism underlying diuretic effect of L-NAME at a subpressor dose. Am J Physiol Renal Physiol 281: F414–F419, 2001.—The diuretic effects of nitric oxide (NO) synthase inhibitors administered at subpressor dose in rats are controversial, and the tubular segments involved are not known. In the present study, we examined the effect of N\textsuperscript{\textbeta}-nitro-L-arginine methyl ester (L-NAME) at a subpressor dose on renal interstitial NO and cGMP activity and on renal tubular segmental reabsorption of fluid in the rat. Intravenous infusion of L-NAME at 1 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) in Sprague-Dawley rats (N = 8), which did not alter mean arterial pressure or glomerular filtration rate, significantly increased urine flow rate (U\textsubscript{v}; from 78.2 ± 12.7 to 117.1 ± 14.9 \( \mu \text{l/min}, P < 0.05 \)). Paradoxically, this effect of L-NAME was concomitant with significant increases in nitrite/nitrate (from 10.79 ± 2.60 \( \mu \text{M}, P < 0.05 \)) and cGMP (from 0.65 ± 0.09 to 0.98 ± 0.18 \( \text{nM}, P < 0.05 \)) concentrations in renal cortical microdialysate as well as the nitrite/nitrate concentration in the medullary microdialysate. Micropuncture studies in the superficial nephron revealed that L-NAME significantly increased the flow rate (from 8.3 ± 0.9 to 12.2 ± 1.2 \( \text{nM/min}, P < 0.05 \)) and fractional delivery of fluid to the distal tubule, but not those in the late proximal tubule. In conclusion, L-NAME, at the subpressor dose used in this study, increased renal nitrate/nitrite and cGMP and inhibited fluid reabsorption in tubular segments between the late proximal tubule and the distal tubule of superficial nephrons.

The physiological role of nitric oxide (NO) in the regulation of renal tubular transport of fluid and sodium is an important question because of its fundamental significance in renal physiology and its implications in disease such as hypertension. Despite intensive study in the last decade, many uncertainties still exist in this area.

One of the most significant controversies in this area arises from the apparent discrepancy between the effect of NO on the tubular transport machinery studied in vitro and the effect of NO synthase (NOS) inhibitors on fluid and sodium reabsorption in vivo. A number of studies have demonstrated that NO, either generated endogenously or released from NO donors, inhibits key transporters in renal tubular epithelial cells (15). For instance, NO has been shown to inhibit Na\textsuperscript{\textbeta}-K\textsuperscript{\textbeta}-AT-Pase or Na\textsuperscript{\textbeta}/H\textsuperscript{\textbeta} exchangers, both playing pivotal roles in tubular fluid and sodium reabsorption in several types of renal tubular epithelial cells (7, 11, 13, 14, 20). If NO serves as a tonic inhibitor of tubular fluid and sodium reabsorption, administration of NOS inhibitors in vivo should decrease fluid and sodium excretion. However, systemic administration of NOS inhibitors has been repeatedly shown to be diuretic and natriuretic (10, 15).

Studies using systemic administration of NOS inhibitors can be roughly divided into two categories, those using NOS inhibitors at doses high enough to increase mean arterial pressure (MAP) and those using subpressor doses. The diuretic and natriuretic effect of NOS inhibitors at pressor doses appears to involve a “pressure natriuresis” mechanism because it is abolished when renal perfusion pressure or renal interstitial hydrostatic pressure is prevented from being increased (8). An interaction with sympathetic nervous activity may also be involved. Thus the diuretic and natriuretic effect of NOS inhibitors at pressor doses, although providing important information regarding blood pressure regulation, may not reflect the direct role that NO might play in tubular reabsorption. Systemic administration of NOS inhibitors at subpressor doses could be more relevant in revealing the role of NO in tubular transport. However, results from studies using this approach are much more controversial. In several studies in humans, intravenous infusion of NOS inhibitors at subpressor doses was shown to decrease urinary excretion of fluid and/or sodium (1, 3, 18). In a study in the rat, however, a bolus injection of the NOS inhibitor N\textsuperscript{\textbeta}-nitro-L-arginine methyl ester (L-NAME) was reported to increase urinary excretion of fluid and sodium without affecting the MAP (24). Controversy also exists in the same species, as Lahera et al. (12) reported that intravenous infusion of L-NAME at subpressor doses decreased urinary excretion of fluid and sodium in rats.

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Two critical questions emerge from these studies. First, it is not clear whether putative NOS inhibitors such as L-NAME at the dose administered truly reduced intrarenal NO activity. The absence of a pressor effect eliminated a confounding factor but at the same time makes the inhibition of NO activity questionable. The recent development of renal interstitial microdialysis technique has made it possible to estimate renal interstitial concentrations of substances (2), including NO and its downstream products (25). Second, although studies have been performed to examine the effect of NOS inhibitors or NO donors on several tubular segments individually (5, 6, 22, 24), the relative contributions of various tubular segments in vivo to the alteration of fluid reabsorption induced by systemic administration of NOS inhibitors at subpressor doses are not clear. Micropuncture studies at multiple nephron sites should provide relevant information in this regard. In the present study, using a combination of clearance, microdialysis, and multiple-site micropuncture techniques, we assessed the effect of systemic administration of L-NAME at a subpressor dose on renal interstitial NO and cGMP activity and on tubular segmental fluid reabsorption in an attempt to fill these significant gaps in our existing knowledge.

MATERIALS AND METHODS

Animal preparation. Male Sprague-Dawley rats, of 260–350 g body wt, were used. Animals were prepared as previously described (19). Briefly, rats were anesthetized with Inactin (100–120 mg/kg body wt ip) and placed on a thermally controlled table to maintain normal body temperature. A tracheostomy was then performed, and oxygen was provided. A PE-50 catheter was inserted into the left carotid artery for blood pressure measurement and blood sample collection. A PE-50 catheter was inserted into the left jugular vein, and a continuous infusion of normal saline containing 2% inulin at a rate of 2% body wt/h was initiated. A catheter was also inserted into the right jugular vein for drug or vehicle administration. A piece of PE-90 tubing was placed in the bladder to collect urine. The left kidney was then exposed by a flank incision. The kidney was dissected free from the surrounding fat and connective tissue. Microdialysis membranes were inserted as described below. The kidney was immobilized in a kidney holder lined with a thin layer of cotton soaked in mineral oil. Care was taken to avoid damaging the renal capsule or stretching the pedicle. The kidney and the incision were covered with cotton soaked in mineral oil, and the animal was allowed ~80 min to recover before the initiation of baseline collections.

Protocols. Two groups of rats were studied. In the L-NAME group, after the recovery period, a 30-min baseline collection of urine and renal cortical and medullary microdialysate and a collection of distal and proximal tubular fluid were taken. Infusion of an L-NAME (Sigma, St. Louis, MO) solution into the right jugular vein was then initiated at a rate of 1 μg L-NAME·kg body wt·min⁻¹ (8 μl/min). Thirty minutes later, a second 30-min experimental collection was taken. At the end of experiment, the animal was killed by air embolism. In the control group, the same protocol was used except that normal saline, the solvent of L-NAME, was infused instead of an L-NAME solution, at a similar infusion rate.

Micropuncture. The recollection free-flow micropuncture technique was utilized to collect tubular fluid from the late proximal tubule and the distal tubule visible on the kidney surface as described previously (19). Briefly, a loop of distal tubule on the kidney surface was identified either by appearance or with a bolus injection of a lissamine green solution. The distal tubule loop was then punctured by a glass micropipette that had been filled at the tip with castor oil stained with Sudan black. A column of the stained castor oil was injected into the tubular lumen, and a timed collection of tubular fluid was initiated. The collection was manipulated in such a way that the oil column remained in position just downstream, adjacent to the pipette tip. Proximal tubules were similarly identified and punctured. The loop of proximal tubule punctured was confirmed to be a late loop of proximal tubule visible on the kidney surface by observing the flow of a stained oil drop. Fluid from one to three distal tubules and one to three proximal tubules was collected in each animal during the baseline collection period. After the administration of either L-NAME or vehicle, collections of tubular fluid from the same tubules were performed. Only successful paired collections were included in the final comparisons.

In vivo microdialysis. Micropuncture technique, as described previously (2), was used to collect renal interstitial fluid for estimation of NO metabolites and cGMP concentrations in the renal cortex and medulla. Microdialysis membranes (5 mm, 20-kDa molecular mass cutoff, Bioanalytical Systems, West Lafayette, IN) connected to an inflow and an outflow tube were placed into the renal cortex and medulla using a 30-gauge needle. The inflow tube was connected to an airtight syringe controlled by a BAS Baby Bee syringe drive (Bioanalytical Systems). The microdialysis membrane was continuously perfused at 2 μl/min with a sterile filtered perfusate containing (in mM) 137 NaCl (cortex) or 500 NaCl (medulla), 3 KCl, 8 Na₂HPO₄, 1.5 KH₂PO₄, 1 CaCl₂, and 0.5 MgCl₂, pH 7.4. The cortical and medullary microdialysate was collected from the outflow tubes. After the animal was killed at the end of the experiment, the kidney was dissected to confirm the correct positioning of the microdialysis membranes.

In vitro microdialysis. Relative recovery rates of nitrate and cGMP were assessed by in vitro microdialysis. Four microdialysis membranes were immersed in a large volume of bulk perfusate solution containing 10 μM nitrate and 10 nM cGMP. The membranes were perfused with the perfusate at 2 μl/min, and the dialysate was collected after an equilibration period of 25 min. Concentrations of nitrate and cGMP in the dialysate and in an aliquot of the bulk solution collected at the end of dialysis were measured. Relative recovery rates of nitrate and cGMP, calculated by dividing their concentrations in the dialysate by those in the bulk solution, were ~60%.

Analysis. Inulin concentrations in plasma and urine were measured by the anthrone method, and the results were used to calculate glomerular filtration rate (GFR). Sodium concentrations in plasma and urine were measured by using an automatic flame photometer (Instrumentation Laboratory, Lexington, MA). The volume of tubular fluid collected was measured using a constant-bore, 1-μl glass capillary tube under a dissecting microscope (19). Inulin concentrations in tubular fluid as well as in plasma were measured by using a fluorescence microinulin method (19) to calculate single-nephron GFR (SNGFR). Nitrite/nitrate concentrations in microdialysate, plasma, and urine were measured by using a sensitive fluorometric method (Cayman, Ann Arbor, MI). Plasma and urine were filtered through a centrifugal filter device (Microcon YM-10, 10,000-Da molecular mass cutoff, Millipore, Bedford, MA) before analysis. cGMP concentrations in microdialysate were measured by using an immuno-
Table 1. Effect of intravenous infusion of L-NAME (at 1 µg·kg⁻¹·min⁻¹) on urinary excretion of water and sodium in rats

<table>
<thead>
<tr>
<th>Group/Period/P</th>
<th>MAP, mmHg</th>
<th>GFR, ml/min</th>
<th>U₁, µl/min</th>
<th>UᵣNaV, µeq/min</th>
<th>FₑNaV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>135 ± 6</td>
<td>2.47 ± 0.29</td>
<td>67.3 ± 12.6</td>
<td>11.65 ± 2.46</td>
<td>3.00 ± 0.45</td>
</tr>
<tr>
<td>Saline</td>
<td>133 ± 4</td>
<td>2.21 ± 0.33</td>
<td>63.2 ± 6.9</td>
<td>12.17 ± 1.74</td>
<td>3.95 ± 0.61</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>L-NAME (N = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>134 ± 6</td>
<td>2.32 ± 0.21</td>
<td>78.2 ± 12.7</td>
<td>10.69 ± 1.72</td>
<td>3.19 ± 0.58</td>
</tr>
<tr>
<td>L-NAME</td>
<td>131 ± 6</td>
<td>2.50 ± 0.22</td>
<td>117.1 ± 14.9</td>
<td>18.67 ± 2.95</td>
<td>5.01 ± 0.69</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. N, L-NAME, N⁵-nitro-L-arginine methyl ester; MAP, mean arterial blood pressure; GFR, glomerular filtration rate; U₁, urine flow rate; UᵣNaV, urinary sodium excretion; FₑNaV, fractional excretion of sodium; NS, not significant. P values are from comparisons between 2 periods in each group.

assay method after acetylation (R&D Systems, Minneapolis, MN).

Statistics. Results are shown as means ± SE. The numbers of animals used are shown as N, whereas the numbers of tubules used are shown as n. Comparisons between two periods within each group were made by using a paired t-test. P < 0.05 was considered statistically significant.

RESULTS

As summarized in Table 1, L-NAME at 1 µg·kg⁻¹·min⁻¹ did not significantly alter the MAP [134 ± 6 mmHg in the baseline period vs. 131 ± 6 mmHg in the L-NAME period, N = 8, not significant (NS)], confirming that this was a subpressor dose. Whereas L-NAME did not significantly change GFR, it significantly increased urine flow rate (U₁) by ~50% (78.2 ± 12.7 vs. 117.1 ± 14.9 µl/min, N = 8, P < 0.05). Absolute and fractional sodium excretion were also significantly increased by ~50–75% during L-NAME infusion (Table 1). None of these parameters changed significantly during vehicle infusion in the control group (Table 1). These data indicate that L-NAME at this dose decreases tubular reabsorption of fluid and sodium in rats.

To determine the effect of L-NAME on intrarenal NO activity, we performed renal microdialysis to sample renal interstitial fluid from the cortex and the medulla. Concentrations of nitrite/nitrate, the stable metabolites of NO, and concentrations of cGMP, the most common mediator of NO effects, were measured in the renal cortical and medullary microdialysate to assess the concentrations of these substances in the renal cortical and medullary interstitial fluid, respectively. Interestingly, and paradoxically, intravenous infusion of L-NAME at this dose significantly increased the concentrations of nitrite/nitrate in renal cortical and medullary microdialysate by ~50% (10.79 ± 1.20 vs. 16.50 ± 2.60 µM from the cortex, and 11.09 ± 1.31 vs. 15.95 ± 2.98 µM from the medulla, N = 8, P < 0.05). Consistent with an increase in NO activity in the renal cortex, the concentration of cGMP in the renal cortical microdialysate was also significantly increased by L-NAME (0.65 ± 0.09 vs. 0.98 ± 0.18 nM, N = 7, P < 0.05). The cGMP concentration in the medullary microdialysate tended to increase during L-NAME infusion, but it did not reach statistical significance (Table 2). Relative changes in nitrite/nitrate and cGMP concentrations in the renal cortical microdialysate during L-NAME infusion were depicted in Fig. 1. Plasma nitrite/nitrate concentrations were not significantly changed by L-NAME, consistent with the absence of systemic inhibition of NO generation and thus the absence of a pressor effect. Urinary nitrite/nitrate was not altered either (Table 2). Because the urinary nitrite/nitrate concentration is probably 10-fold higher than that in the renal interstitial fluid, the moderate increase of nitrite/nitrate concentration in renal interstitial fluid in this study might not be sufficient to be reflected in the urinary concentration of these compounds. All of these parameters remained unchanged during the vehicle infusion in the control group (Table 2).

Table 2. Effect of intravenous infusion of L-NAME (at 1 µg·kg⁻¹·min⁻¹) on nitrite/nitrate and cGMP concentrations in renal cortical and medullary microdialysate and on nitrite/nitrate concentrations in plasma and urine

<table>
<thead>
<tr>
<th>Group/Period/P</th>
<th>Cortical Nitrite/Nitrate, µM</th>
<th>Medullary Nitrite/Nitrate, µM</th>
<th>Cortical cGMP, nM</th>
<th>Medullary cGMP, nM</th>
<th>Plasma Nitrite/Nitrate, µM</th>
<th>Urinary Nitrite/Nitrate, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 7)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9.67 ± 1.12</td>
<td>11.03 ± 1.16</td>
<td>0.62 ± 0.11</td>
<td>1.14 ± 0.23</td>
<td>260 ± 14</td>
<td>330 ± 35</td>
</tr>
<tr>
<td>Saline</td>
<td>12.51 ± 1.17</td>
<td>12.28 ± 2.08</td>
<td>0.85 ± 0.13</td>
<td>1.76 ± 0.53</td>
<td>249 ± 15</td>
<td>339 ± 45</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L-NAME (N = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>10.79 ± 1.20</td>
<td>11.09 ± 1.31</td>
<td>0.65 ± 0.09</td>
<td>3.06 ± 0.60</td>
<td>262 ± 19</td>
<td>276 ± 18</td>
</tr>
<tr>
<td>L-NAME</td>
<td>16.50 ± 2.60</td>
<td>15.95 ± 2.98</td>
<td>0.98 ± 0.18</td>
<td>4.91 ± 0.97</td>
<td>266 ± 21</td>
<td>299 ± 14</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. P values are from comparisons between 2 periods in each group.
Recollection free-flow micropuncture of the superficial nephron was performed to determine the tubular segment(s) involved in the above tubular effect of L-NAME. As shown in Table 3, SNGFR, calculated from either the proximal collections or the distal collections, was not significantly changed by L-NAME. SNGFR calculated from the proximal collections were higher or tended to be higher than those calculated from the distal collections, probably caused by the tubularglomerular feedback effect during proximal collections. The tubular fluid flow rate in the late surface proximal tubule and the fractional delivery of fluid to this point did not change significantly during L-NAME infusion, although there was a tendency for them to increase (Table 3). In contrast, the tubular fluid flow rate in the surface distal tubule was significantly increased by 10.220.33.4 on June 23, 2017 http://ajprenal.physiology.org/ Downloaded from

DISCUSSION

There are two major novel findings in the present study. First, the diuretic and natriuretic effect of L-NAME intravenously infused at 1 μg·kg⁻¹·min⁻¹ in rats was concomitant with an increase of NO and cGMP activity in the renal interstitium. Second, infusion of L-NAME intravenously at 1 μg·kg⁻¹·min⁻¹ in rats was concomitant with an increase of NO and cGMP activity in the renal interstitium. The important comparisons in this study were paired comparisons made within each group. The relative changes in fluid flow rates along the nephron during vehicle or L-NAME infusion expressed as percentages of corresponding baselines are depicted in Fig. 2. SNGFR obtained from proximal and distal collections were pooled to calculate changes of flow rates of glomerular filtrate shown in this figure.
sion of L-NAME at the dose used did not significantly alter the fluid flow rate at the late surface proximal tubule but significantly increased the fluid flow rate at the puncture site of the distal tubule to an extent similar to the increase of $U_v$.

Systemic administration of L-NAME at doses similar to that used in the present study was shown to increase tubular reabsorption of fluid and/or sodium in humans (1, 3, 18), which could be interpreted as a reflection of a tonic inhibitory effect of NO on tubular reabsorption. In rats, systemic administration of L-NAME at subpressor doses has been shown to either increase (24) or decrease (12) tubular reabsorption of fluid and sodium, leading to suggestions of opposite effects of NO on tubular reabsorption. In the present study, intravenous infusion of L-NAME at a subpressor dose was shown to be diuretic and natriuretic. However, our data indicate that the diuretic and natriuretic effect of L-NAME at this dose is not likely due to an inhibition of intrarenal NO activity because intrarenal NO activity measured by microdialysis was in fact increased by L-NAME. Because of the lack of more specific methods to manipulate NO activity, it is unclear whether the effect of L-NAME on tubular transport observed in this study is a direct result of the paradoxical increase of intrarenal NO activity or is caused by some unknown actions of L-NAME. Nevertheless, the association of an increased intrarenal NO activity and the diuretic and natriuretic effect of L-NAME does suggest that the increase of intrarenal NO activity might contribute to the inhibition of fluid and sodium reabsorption caused by L-NAME. This possibility is strongly supported by in vitro studies demonstrating that NO inhibits the machinery of tubular reabsorption such as Na$^+$/K$^+$-ATPase (7, 11, 13) and Na$^+$/H$^+$ exchanger (20) activities. Thus, our data indicate that the diuretic and natriuretic effect of systemic administration of L-NAME at subpressor dose in rats might be caused by a paradoxical increase in intrarenal NO activity and favors the notion that NO has a tonic inhibitory effect on renal tubular fluid and sodium reabsorption.

That acute administration of an NOS inhibitor in vivo results in an increase of detectable NO is unusual but not unprecedented. In a study by Losonczy et al. (16), it was shown that L-NAME increased urinary excretions of nitrite/nitrate and cGMP in rats that were anesthetized and underwent acute surgical preparation. In the present study, the renal interstitial nitrite/nitrate concentration was increased by L-NAME, at a dose at least 30-fold lower than that used in the Losonczy study, without the increase significantly affecting plasma or urinary nitrite/nitrate concentrations. Thus the increase of renal interstitial nitrite/nitrate is unlikely due to an increased reabsorption of nitrite/nitrate as proposed by others (23). Moreover, cGMP concentration in the renal cortical interstitium was also significantly increased by L-NAME. cGMP is the most common mediator of NO effects and is specifically involved in several effects of NO on tubular reabsorption (14, 17, 20, 24). Taken together, these data indicate that it was likely that intrarenal NO activity was indeed increased by L-NAME in the present study. In a microdialysis study by Zou and Cowley (25), L-NAME was shown to moderately decrease nitrite/nitrate concentration in renal microdialysate by $\sim$20%. However, the dose of L-NAME used in that study was 10-fold higher than ours. In addition, our ability to measure decreases of renal interstitial NO activity was confirmed by our previous studies in which intravenous infusion of $N^\omega$-monomethyl-L-arginine, another putative NOS inhibitor, at 500 $\mu$g kg$^{-1}$, min$^{-1}$ (a pressor dose) decreased rat renal interstitial nitrite/nitrate concentrations by 16–18%. It is not clear how L-NAME increased NO activity in the kidney as shown in the present study. It is known that L-NAME and $N^\omega$-nitro-L-arginine, its putative active form, have a much higher affinity for endothelial NOS compared with inducible NOS (21). One possibility is that L-NAME at the low dose used in the present study might bind to a certain pool of endothelial NOS and shift the natural substrate L-arginine to inducible NOS possibly present in the kidney, thereby increasing intrarenal NO generation. More studies are needed to test this and other possibilities.

NO donors or inhibitors have been shown to affect fluid and/or electrolyte transport in several tubular segments studied individually and in cells with characteristics of various tubular segments. These tubular segments include the proximal tubule (4, 5, 24), the thick ascending limb (11), and the collecting duct (6, 22). In the present study, L-NAME at the dose used did not significantly affect the flow rate of tubular fluid by the late proximal tubule in superficial nephrons. However, the fluid flow rate in the surface distal tubule was significantly increased by $\sim$50%, which was similar to the increase of final $U_v$. Therefore, the tubular segments of the superficial nephron where the fluid reabsorption is affected by L-NAME are likely between the late proximal tubule and the distal tubule. For the same reason as discussed above, it is possible, although inconclusive, that the inhibition of fluid reabsorption in this part of the nephron is due to a moderate increase of intrarenal NO activity. This suggests that NO might play a tonic role in the regulation of fluid transport in this part of nephron. It is noteworthy that cGMP was significantly increased in the cortex but not in the medulla. In the meantime, there is abundant evidence for a significant effect of NO on the transport machinery of proximal tubular cells (15). Thus the S2 and S3 segments of the proximal tubule appear to be the likely targets for NO regulation under physiological conditions. It is important to note that only superficial nephrons were punctured in the present study. Because of nephron heterogeneity in fluid and sodium handling (9), caution should be taken when one is extrapolating these findings to deep nephrons.

Our results did not rule out possible roles of NO in the regulation of fluid and electrolyte transport in other tubular segments. The regulation of tubular transport by NO is very likely to be a complex process involving a variety of interactions between NO and other regulatory mechanisms of tubular transport (15).
Under physiological conditions, tubular segments between the late proximal tubule and the distal tubule might be the primary targets for NO. When other regulatory mechanisms undergo adjustments under situations such as hypertension, the NO mechanism might be recruited in other tubular segments, and the overall role of NO might be altered. Therefore, understanding the differential roles that NO plays in tubular transport under diverse circumstances is critical to our understanding of renal physiology as well as the development of disease such as hypertension. In this regard, the results of the present study are of both physiological and pathophysiological significance.

In conclusion, the diuretic and natriuretic effect of L-NAME at the subpressor dose used in the present study is concomitant with an increase in intrarenal NO and cGMP activity. In superficial nephrons, the inhibition of fluid reabsorption by this dose of L-NAME most likely occurs in tubular segments between the late proximal tubule and the distal tubule.

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REFERENCES


