Role of Nitric Oxide in Renal Papillary Blood Flow and Sodium Excretion


Renal medullary interstitial infusion of \( N^G \)-nitro-l-arginine (120 \( \mu \)g/hr, \( n=7 \)) decreased papillary blood flow to 71\( \pm \)5% of control without altering outer cortical flow. Before \( N^G \)-nitro-l-arginine infusion, interstitial acetylcholine administration (200 \( \mu \)g/hr) increased cortical and papillary blood flow to 134\( \pm \)6% and 113\( \pm \)2% of control, respectively. After \( N^G \)-nitro-l-arginine administration, the vasodilator response to acetylcholine was abolished. In clearance experiments, renal medullary infusion of \( N^G \)-nitro-l-arginine (120 \( \mu \)g/hr, \( n=1 \)) significantly decreased total renal blood flow by 10%, renal interstitial fluid pressure by 23%, sodium excretion by 34%, and urine flow by 39% without altering glomerular filtration rate, fractional sodium and water excretion, blood pressure, or urine osmolality. These data indicate that selective inhibition of nitric oxide in the renal medullary vasculature reduces papillary blood flow, which is associated with decreased sodium and water excretion. We conclude that nitric oxide exerts a tonic influence on the renal medullary circulation.

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Methods

Surgical Preparation

Experiments were performed on 24 male Sprague-Dawley rats purchased from SASCO, Inc., Madison, Wis. All procedures performed on the animals were approved by the Medical College of Wisconsin Animal Care Committee. Rats were anesthetized with Inactin (100 mg/kg i.p.) and placed on a heated table to maintain body temperature at 37\(^\circ\)C. Cannulas were placed in the femoral artery for measurement of mean arterial pressure (MAP), in the jugular vein for infusions, and in the left and right ureters for urine collection. An extruded piece of PE-10 was inserted through the cortex from the lateral border of the kidney into the renal medullary interstitium with its tip at the border of the inner and outer medulla. The placement of the catheter was confirmed at the end of each study by infusion of lissamine green dye into the catheter and viewing of the tip after postmortem hemisection of the kidney. Isotonic saline (or drug) was infused into the renal medullary interstitium at a rate of 0.5 ml/hr throughout each protocol. All experiments were performed in rats undergoing a mild saline diuresis by intravenous infusion of 1% bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.) in saline at a rate of 1 ml/hr/100 g body wt. This model was chosen because preliminary experiments showed inconsistent renal excretory responses to L-NA in hydropenic or euvoletic animals. The rats were studied in the protocols described below.

Protocol 1: Effect of \( N^G \)-Nitro-l-Arginine on Renal Cortical and Papillary Blood Flow

One week before the acute experiment, a small amount of renal cortical tissue on the dorsal surface of the left kidney was removed to allow for exposure of the renal papilla as previously described.\(^8\) On the day of the experiment, rats were surgically prepared as described above. In addition, the left kidney was placed in a holder, and the papilla was exposed by excision of the ureter. Cortical and papillary blood flows were measured with a laser Doppler flowmeter (model Pf1, Perimed KB, Stockholm). Cortical and papillary blood flows are expressed as a percentage of the respective laser Doppler flow signal measured during the control period.

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From the Department of Physiology, Medical College of Wisconsin, Milwaukee, Wis.

Address for correspondence: David L. Mattson, Department of Physiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.
Group 1: Time control (n=5). Renal cortical and papillary blood flow was measured during four sequential 1-hour periods. Saline vehicle was continuously infused into the renal medullary interstitium throughout the entire experiment.

Group 2: Effects of renal medullary interstitial infusion of N^G^-nitro-L-arginine on renal cortical and papillary blood flow (n=7). Renal cortical and papillary blood flow, MAP, and hematocrit were measured during a 30-minute control period in which vehicle was infused into the renal medullary interstitium. Blood flow in the cortex and medulla was again measured during the final 15 minutes of a 75-minute interstitial infusion of L-NA (120 μg/hr). Blockade of NO was confirmed by measurement of the cortical and papillary blood flow response to acetylcholine (200 μg/hr) before and after L-NA treatment. Blood flow was allowed to return to control levels in a postcontrol period after each acetylcholine infusion period. In preliminary experiments, this dose of L-NA was determined to selectively alter papillary blood flow without changing MAP or cortical blood flow.

Group 3: Effects of injection of N^G^-nitro-L-arginine into the renal artery on cortical and papillary blood flow (n=5). To determine if the interstitial effects of L-NA were due to recirculation, we infused the same dose of L-NA directly into the renal artery of a separate group of rats. Preliminary experiments demonstrated that intrarenal arterial infusion of acetylcholine at a rate of 200 μg/hr, the dose infused into the interstitium, would decrease blood pressure dramatically. For this reason, the intrarenal arterial infusion of acetylcholine used to check NO blockade by L-NA was reduced to 20 μg/hr in this group of rats. The L-NA dose was the same for both renal arterial and renal interstitial infusions (120 μg/hr). The renal artery was cannulated in this group of rats by placement of a 30-gauge needle across the abdominal aorta with its tip in the initial portion of the renal artery. The needle was held in place by gluing it to the aorta with cyanoacrylate adhesive; a small piece of abdominal fat was used to anchor the needle in place. The intrarenal arterial infusion rate was maintained at 1.0 ml/hr throughout the experiment. Renal cortical and papillary blood flows were measured during a 30-minute control period in which vehicle was infused into the renal artery. Blood flow, MAP, and hematocrit were again measured during the final 15 minutes of a 75-minute renal arterial infusion of L-NA (120 μg/hr). Blockade of NO was confirmed by measurement of the cortical and papillary blood flow response to acetylcholine (20 μg/hr) before and after L-NA treatment.

Protocol 2: Effect of Medullary Interstitial Infusion of N^G^-Nitro-L-Arginine on Sodium and Water Excretion

Experiments were performed in seven rats prepared for clearance experiments as described above. In addition, a 2-mm flow probe was placed around the left renal artery for measurement of renal blood flow with an electromagnetic flowmeter (model 501, Carolina Medical Electronics, Inc., King, N.C.). A polyethylene capsule was acutely implanted in the renal cortex for measurement of renal interstitial fluid pressure as described previously. [3H]Inulin was included in the infusion solution for measurement of glomerular filtration rate.

In these experiments, renal function was assessed during two 20-minute control periods. L-NA (120 μg/hr) was then infused into the medullary interstitium, and after an hour of equilibration, urine and plasma samples were again collected during two 20-minute experimental clearance periods. Data are presented as mean values for the control and experimental states.

Analytical Methods

Urine flow was determined gravimetrically. Sodium concentration in the samples was determined with a flame photometer (model 143, Instrumentation Laboratories, Lexington, Mass.). Urine osmolality was determined with a freezing point depression osmometer (model 5004, Precision Instruments, Sudbury, Mass.). Systemic arterial protein concentration was determined with a refractometer. [3H]Inulin concentration of the samples was determined with a liquid scintillation counter (model 2450, Packard Instrument Co. Inc., Downers Grove, Ill.). Glomerular filtration rate was calculated by multiplication of the urine-to-plasma [3H]inulin counts per minute ratio by the urine flow rate. Urine flow, sodium excretion, renal blood flow, and glomerular filtration rate were all normalized per gram kidney weight.

Statistical Methods

Data are presented as mean ± SEM. The significance of differences in multiple measured values was evaluated with an analysis of variance for repeated measures and a Duncan multiple range test. A paired t test was used where applicable. A value of p<0.05 was considered significant.

Results

Protocol 1

Time control (group 1, n=5). Cortical and papillary blood flows were not significantly altered during the 4-hour time control experiment. MAP was 95±4 mm Hg, and hematocrit was 41±0.5% in the control period; neither changed during the experimental protocol.

Effects of renal medullary interstitial infusion of N^G^-nitro-L-arginine on renal cortical and papillary blood flow (group 2, n=7). Renal medullary interstitial infusion of L-NA significantly decreased papillary blood flow by 24±4% of control but did not alter superficial cortical blood flow (Figure 1, top panel). Before medullary interstitial L-NA administration, interstitial acetylcholine infusion significantly increased (p<0.05) both cortical and papillary blood flow to 34±6% and 13±2% of control, respectively. After L-NA infusion, the response to acetylcholine was significantly blunted (p<0.05); acetylcholine infusion only increased cortical flow by 6±14% and papillary blood flow by 15±44% of the acetylcholine response elicited before L-NA infusion (Figure 1, bottom panel). MAP averaged 114±5 mm Hg during the control period and fell (p<0.05) to 101±5 mm Hg during acetylcholine infusion. It returned to levels not significantly different from control after acetylcholine and remained unaltered for the remainder of the experiment. Hematocrit was 45±1% in the control.
Blood Flow (n=7)

Protocol 2: Effect of Medullary Interstitial Infusion of \( N^G \)-Nitro-L-arginine (120 \( \mu \)g/hr) on Renal Function in Infused and Contralateral Kidney

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Control</th>
<th>( N^G )-Nitro-L-arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused kidney (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( U_{Na}V ) (( \mu )eq/min/gkwt)</td>
<td>4.7±1.0</td>
<td>3.1±0.7*</td>
</tr>
<tr>
<td>UV (( \mu )l/min/gkwt)</td>
<td>35.6±5.1</td>
<td>21.7±3.6*</td>
</tr>
<tr>
<td>Uosm (mosm/kg H2O)</td>
<td>413±15</td>
<td>460±27</td>
</tr>
<tr>
<td>FE ( Na ) (%)</td>
<td>2.9±0.6</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td>FE ( H_2O ) (%)</td>
<td>3.1±0.4</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>GFR (ml/min/gkwt)</td>
<td>1.1±0.04</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>RBF (ml/min/gkwt)</td>
<td>8.6±0.7</td>
<td>7.7±0.7*</td>
</tr>
<tr>
<td>FF (%)</td>
<td>26±2</td>
<td>24±2</td>
</tr>
<tr>
<td>RISFP (mm Hg)</td>
<td>10.2±1.1</td>
<td>7.8±1.3*</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>119±2.4</td>
<td>119±2.4</td>
</tr>
<tr>
<td>Contralateral kidney (n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( U_{Na}V ) (( \mu )eq/min/gkwt)</td>
<td>3.0±0.7</td>
<td>3.1±0.7</td>
</tr>
<tr>
<td>UV (( \mu )l/min/gkwt)</td>
<td>22.4±4.4</td>
<td>17.9±3.2</td>
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<tr>
<td>Uosm (mosm/kg H2O)</td>
<td>528±39</td>
<td>561±51</td>
</tr>
<tr>
<td>FE ( Na ) (%)</td>
<td>1.6±0.4</td>
<td>1.7±0.4</td>
</tr>
<tr>
<td>FE ( H_2O ) (%)</td>
<td>1.8±0.3</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>GFR (ml/min/gkwt)</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
</tr>
</tbody>
</table>

 Values are mean±SEM. \( U_{Na}V \), sodium excretion; gkwt, gram kidney weight; UV, urine flow rate; Uosm, urine osmolality; FE\( Na \), fractional sodium excretion; FE\( H_2O \), fractional water excretion; GFR, glomerular filtration rate; RBF, renal blood flow; FF, filtration fraction; RISFP, renal interstitial fluid pressure; MAP, mean arterial pressure. *p<0.05 from control.

The present study evaluated the effects of blockade of NO production in the renal medulla on cortical and papillary blood flow and sodium and water excretion. Renal medullary interstitial infusion of L-NA, a NO inhibitor, selectively reduced papillary blood flow without altering superficial cortical blood flow or MAP. The fall in papillary blood flow was associated with a reduction in renal interstitial fluid pressure and sodium and water excretion. Blockade of NO was confirmed by evaluation of the cortical and papillary blood flow response to medullary interstitial acetylcholine infusion. L-NA completely blocked the increase in papillary blood flow produced by acetylcholine infusion, indicating that medullary production of NO was blocked in the present experiments.

To determine if the effects of interstitial L-NA were due to recirculation, we studied the effect of renal arterial infusion of L-NA. Intrarenal arterial infusion of L-NA decreased both cortical and papillary blood flow, significantly decreased (p<0.05) sodium excretion, urine flow rate, renal blood flow, and renal interstitial fluid pressure in the infused kidney by 34%, 39%, 10%, and 23%, respectively. There were no significant alterations in urine osmolality, fractional sodium and water excretion, glomerular filtration rate, filtration fraction, MAP, or hematocrit in the infused kidney during interstitial L-NA infusion. In the noninfused kidney, there was no change in sodium excretion, urine flow rate, fractional sodium and water excretion, urine osmolality, or glomerular filtration rate during L-NA infusion.

**Discussion**

The effects of NO production in the renal medulla on cortical and papillary blood flow and sodium and water excretion are significant. Renal medullary interstitial infusion of L-NA, a NO inhibitor, selectively reduced papillary blood flow without altering superficial cortical blood flow or MAP. The fall in papillary blood flow was associated with a reduction in renal interstitial fluid pressure and sodium and water excretion. Blockade of NO was confirmed by evaluation of the cortical and papillary blood flow response to medullary interstitial acetylcholine infusion. L-NA completely blocked the increase in papillary blood flow produced by acetylcholine infusion, indicating that medullary production of NO was blocked in the present experiments.

**Protocol 2: Effect of Medullary Interstitial Infusion of \( N^G \)-Nitro-L-arginine on Sodium and Water Excretion, Renal Interstitial Fluid Pressure, and Whole-Kidney Blood Flow (n=7)**

Plasma protein concentration averaged 4.8±0.1 g/dl, plasma osmolality 299±4 mosm/l, and plasma sodium concentration 140±1 meq/l in these animals. Table 1 summarizes clearance data in the infused and contralateral kidney. Medullary interstitial L-NA infusion

**Figure 1.** Top panel: Line plot shows effect of renal medullary interstitial infusion of \( N^G \)-nitro-L-arginine (L-NA) (120 \( \mu \)g/hr) on cortical and papillary blood flow. Bottom panel: Bar graph shows effect of interstitial L-NA infusion on cortical and papillary blood flow response to acetylcholine (ACh) infusion (200 \( \mu \)g/hr). * Significant difference (p<0.05) from previous point.

Effects of intrarenal arterial infusion of \( N^G \)-nitro-L-arginine on cortical and papillary blood flow (group 3, n=5). Infusion of acetylcholine (20 \( \mu \)g/hr) selectively increased (p<0.05) cortical blood flow to 117±1% of control, whereas papillary blood flow was unaltered. Blood flow in both the cortex and medulla was not different from control during the postcontrol period. Intrarenal arterial infusion of L-NA (120 \( \mu \)g/hr) significantly reduced (p<0.05) both cortical and papillary blood flow to 76±6% and 66±3% of control, respectively. Infusion of acetylcholine after L-NA had no effect on either cortical or papillary blood flow. Neither MAP nor hematocrit was altered from control levels of 95±2 mm Hg and 38±1%, respectively, throughout the experiment.
whereas medullary interstitial infusion of L-NA selectively decreased papillary blood flow. This suggests that the effects of interstitial L-NA are not due to recirculation of the compound.

Although the infusion of acetylcholine was used solely to test whether L-NA blocked the actions of NO, we were surprised to observe that interstitial infusion of acetylcholine increased both cortical and papillary blood flow, whereas NO inhibition with L-NA selectively decreased papillary blood flow. It is possible that medullary interstitial infusion of acetylcholine stimulates NO from portions of the kidney that do not normally release NO. This could explain how interstitial infusion of acetylcholine increased cortical blood flow, whereas interstitial L-NA infusion had no effects other than blocking acetylcholine-induced vasodilation. A second confounding observation is that interstitial acetylcholine infusion increased both cortical and papillary blood flow, whereas infusion of acetylcholine into the renal artery increased only cortical flow. This could be related to the fact that the dose of acetylcholine infused into the renal artery was only one tenth of that infused into the medullary interstitium.

The mechanism by which L-NA decreased sodium and water excretion is unclear. Possible explanations are direct effects on the renal vasculature, direct effects on tubular transport, or indirect effects on tubular transport. Interstitial infusion of L-NA into the medulla did not alter superficial cortical blood flow, but it did reduce papillary blood flow and whole-kidney renal blood flow. The decrease in sodium and water excretion after L-NA infusion could have been caused by the direct effect of L-NA to selectively decrease blood flow and glomerular filtration in the juxtamedullary nephrons. Inhibition of NO could also have altered sodium and water excretion by altering tubular transport. Cyclic GMP, the intracellular mediator of NO, has direct effects on tubular transport.10,11 Alternatively, L-NA treatment could have altered tubular transport by decreasing renal medullary blood flow, which may indirectly influence tubular sodium and water transport by reducing renal interstitial fluid pressure or by altering the renal cortical-medullary solute gradient.12 Because there were no significant effects on glomerular filtration rate, fractional sodium or water excretion, or urine osmolality in the present study, it is difficult to determine the mechanism of the sodium and water retention.

In summary, renal medullary interstitial infusion of L-NA decreases medullary blood flow, sodium excretion, urine flow rate, renal blood flow, and renal interstitial fluid pressure. These studies indicate that the local production of NO in the renal medulla plays a role in the control of papillary blood flow and that a selective reduction in papillary blood flow is associated with sodium and water retention.

References

Role of nitric oxide in renal papillary blood flow and sodium excretion.
D L Mattson, R J Roman and A W Cowley, Jr

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