Responses to Acute Changes in Arterial Pressure on Renal Medullary Nitric Oxide Activity in Dogs

Dewan S.A. Majid, Karim E. Said, Sophia A. Omoro

Abstract—A direct relationship between renal arterial pressure (RAP) and cortical tissue nitric oxide (NO) activity in the canine kidney was reported earlier. We have conducted further experiments to evaluate medullary NO responses to alterations in RAP with the use of a NO-selective microelectrode inserted into the renal medulla of 6 anesthetized, sodium-replete dogs. Graded reductions in RAP (from 140 to 80 mm Hg) elicited decreases in medullary tissue NO concentration, with a slope of 10.2±4.5 nmol · L⁻¹ · mm Hg⁻¹. These changes in NO levels were associated with decreases in urinary excretion rate of nitrate and nitrite (U_NOx_V; control value, 1.7±0.03 nmol · min⁻¹ · g⁻¹; slope, 0.02±0.004 nmol · min⁻¹ · g⁻¹ · mm Hg⁻¹) and sodium excretion (U_Na_V; control, 3.2±0.7 μmol · min⁻¹ · g⁻¹; slope, 0.06±0.02 μmol · min⁻¹ · g⁻¹ · mm Hg⁻¹) without changes in glomerular filtration rate control (0.84±0.06 mL · min⁻¹ · g⁻¹) and sodium excretion (U_Na_V; control, 3.2±0.7 μmol · min⁻¹ · g⁻¹; slope, 0.06±0.02 μmol · min⁻¹ · g⁻¹ · mm Hg⁻¹) without changes in glomerular filtration rate control (0.84±0.06 mL · min⁻¹ · g⁻¹). Intra-arterial administration of the NO synthase inhibitor N⁵-nitro-L-arginine (NLA; 50 μg · kg⁻¹ · min⁻¹) decreased medullary NO concentration by 218±55 nmol · L⁻¹ (n=5) and attenuated the relationship between RAP and NO concentration (slope, 2.7±2.2 nmol · L⁻¹ · mm Hg⁻¹). NLA infusion decreased U_NOx_V (0.8±0.06 nmol · min⁻¹ · g⁻¹) and U_Na_V (1.1±0.2 μmol · min⁻¹ · g⁻¹) without changes in glomerular filtration rate and attenuated RAP versus U_NOx_V and U_Na_V relationships. Total and regional blood flows, as measured by electromagnetic and laser Doppler flowmetry probes, respectively, remained autoregulated both before and during NLA infusion. These data support the hypothesis that acute changes in RAP elicit changes in intrarenal NO production, which may participate in the mediation of pressure natriuresis. (Hypertension. 1999;34[part 2]:832-836.)

Key Words: natriuresis ■ nitric oxide ■ selective electrode ■ laser Doppler flowmetry

It is well appreciated that endogenous nitric oxide (NO) is an important regulator of renal hemodynamics and renal function. Evidence accumulated during recent years indicates that NO exerts a substantive role in regulating tubular sodium reabsorption and in mediating renal arterial pressure (RAP)-induced changes in urinary sodium excretion (U_Na_V). Changes in U_Na_V during acute alterations in RAP within autoregulatory range have been demonstrated to be associated with parallel changes in the urinary excretion rate of NO metabolites nitrate and nitrite (U_NOx_V). More recently, we have also demonstrated a direct relationship between RAP and cortical tissue NO activity with a NO-selective microelectrode in the canine kidney. However, studies have suggested that the deeper nephrons rather than the superficial cortical nephrons are primarily involved in pressure-induced natriuretic responses. Thus, it is possible that medullary rather than cortical tissue NO activity is of more direct interest in relation to the mechanism of pressure natriuresis.

In the present study, we evaluated the responses to alterations in RAP on tissue NO activity in the renal medulla of anesthetized dogs. An NO-selective microelectrode was used to assess the changes in renal tissue NO activity in vivo as shown previously.

Methods

Experiments were performed in 6 mongrel dogs (17 to 21 kg body wt) of either sex supplied by an accredited dealer (Martin Creek Kennels, Williford, Ark). All procedures that involved animals were reviewed and approved by the Animal Care and Use Committee of Tulane University, New Orleans, La. To achieve a positive sodium-replete state, dogs received supplemental amounts of sodium chloride (1.5 g/kg body wt per day for 3 days) in the normal laboratory diet. On the morning of the experiment, the dogs were anesthetized with pentobarbital sodium (30 mg/kg body wt IV); surgical anesthesia was maintained throughout the experiments by additional doses of pentobarbital sodium as needed. Auffed endotracheal tube was inserted into the trachea to allow positive pressure ventilation with an artificial respirator at a breath rate of 18 per minute and a stroke volume of ~15 mL/kg body wt. Body temperature was measured continuously by a telethermometer placed in the rectum of the dog and was maintained within normal range with an electric heating pad placed under the dog. Systemic arterial pressure of the dogs was measured by means of a catheter placed in the abdominal aorta, introduced through the right femoral artery. The catheter was connected to a pressure transducer, and systemic arterial pressure was recorded on a polygraph (model 7D, Grass Instruments). The left femoral artery was cannulated for collection of blood samples. The femoral and jugular veins were cannulated for administration of saline (0.9%; 0.025 mL · min⁻¹ · kg⁻¹), inulin solution, and additional doses of pentobarbital sodium as necessary.

The left kidney was exposed through a flank incision and was denervated by cutting the renal nerves. An electromagnetic flow...
Figure 1. In vitro calibration curve of the NO electrode (n = 4). Doses of the NO donor SNAP were used for calibration. Equivalent NO concentrations of SNAP doses are also depicted on the x axis.

An NO-selective microelectrode (Inter Medical Co, Japan, Ltd) was used to measure the dynamic changes in tissue NO concentration in the kidney as described earlier. Briefly, this is a platinum-iridium alloy electrode (200 μm in diameter) covered with an NO-selective membrane. The reference electrode is composed of carbon fibers. This polarographic electrode measures the current induced by electrochemical oxidation of NO at the electrode surface. Electrodes were precalibrated in vitro by adding known doses of the NO donor compound SNAP to a cuvette in which they were immersed. Figure 1 illustrates the calibration curve generated from the 4 electrodes used in this study. As also noted previously, variations occur in the dose-response relationship among the electrodes, as reflected by the wide standard deviation of the mean current values generated by the SNAP concentrations. To measure tissue NO concentration in the renal medulla, the electrode was inserted to a depth of 13 to 15 mm, depending on the size of the kidney. The positions of the tips of the needle probes were confirmed at the end of each experiment by dissecting the kidney and viewing the needle tract and the regions surrounding the fiber tip. These flow probes were calibrated with a standard calibration device with a motility standard as described previously. An NO-selective microelectrode (Inter Medical Co, Japan) was used to measure the dynamic changes in tissue NO concentration in the kidney as described earlier. Briefly, this is a platinum-iridium alloy electrode (200 μm in diameter) covered with an NO-selective membrane. The reference electrode is composed of carbon fibers. This polarographic electrode measures the current induced by electrochemical oxidation of NO at the electrode surface. Electrodes were precalibrated in vitro by adding known doses of the NO donor compound SNAP to a cuvette in which they were immersed. Figure 1 illustrates the calibration curve generated from the 4 electrodes used in this study. As also noted previously, variations occur in the dose-response relationship among the electrodes, as reflected by the wide standard deviation of the mean current values generated by the SNAP concentrations. To measure tissue NO concentration in the renal medulla, the electrode was inserted to a depth of 13 to 15 mm, depending on the size of the kidney. The positions of the tips of the needle probes were confirmed at the end of each experiment by dissecting the kidney and viewing the needle tract and the regions surrounding the fiber tip. These flow probes were calibrated with a standard calibration device with a motility standard as described previously.

Results

Responses to Reductions in RAP in Medullary Tissue NO Concentration

The Table summarizes and Figure 2 illustrates the mean values obtained in 6 dogs. The mean output current recorded from the electrodes in renal medulla was 8040±1580 pA (n = 6). During graded reductions in RAP from the spontaneous level of 133±6.5 mm Hg to 106.2±4.9 and 79±3.1 mm Hg, decreases in output current (−817±140 and −1734±419 pA, respectively, Figure 2A) were noted from the NO electrode. Figure 2B shows the estimated decreases in medullary tissue NO concentration (−176±30 and −372±90 nmol · L−1, respectively) with in vitro calibration curves for each electrode. Associated changes occurred in UNOx and UNaV during reduction in RAP (Figure 3). UNoV decreased from a control value of 1.7±0.3 to 1.3±0.2 and 0.6±0.2 nmol · min−1 · g−1 and UNaV decreased from 3.2±0.7 to 2.1±0.6 and 0.9±0.3 μmol · min−1 · g−1 during these step reductions in RAP. Urine flow also showed usual decreases during reductions in RAP without changes in total or regional blood flows or GFR (Table).
Renal Responses to Reductions in RAP Before and During Intra-Arterial Administration of NLA

<table>
<thead>
<tr>
<th>RAP Levels, mm Hg</th>
<th>Spontaneous Level (Control)</th>
<th>1st Step Reduction</th>
<th>2nd Step Reduction</th>
<th>Spontaneous Level (Control)</th>
<th>1st Step Reduction</th>
<th>2nd Step Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before NLA</td>
<td>133 ± 6.5</td>
<td>106 ± 2.4</td>
<td>79 ± 3.1</td>
<td>During NLA</td>
<td>137 ± 6.1</td>
<td>113 ± 3.4</td>
</tr>
<tr>
<td>Renal vascular resistance, mm Hg · mL⁻¹ · min⁻¹ · g⁻¹</td>
<td>29.9 ± 2.0</td>
<td>23.2 ± 1.5</td>
<td>17.7 ± 1.6</td>
<td>41.9 ± 4.3*</td>
<td>38.8 ± 5.4</td>
<td>33.1 ± 6.3</td>
</tr>
<tr>
<td>Total renal blood flow, mL · min⁻¹ · g⁻¹</td>
<td>4.5 ± 0.3</td>
<td>4.7 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>3.3 ± 0.3*</td>
<td>3.1 ± 0.3</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>Cortical blood flow, % of the control (n=4)</td>
<td>100</td>
<td>105 ± 6</td>
<td>111 ± 6</td>
<td>91 ± 5</td>
<td>88 ± 5</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>Medullary blood flow, % of the control (n=5)</td>
<td>100</td>
<td>109 ± 4</td>
<td>109 ± 4</td>
<td>88.3 ± 3*</td>
<td>87 ± 7</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>Glomerular filtration rate, mL · min⁻¹ · g⁻¹</td>
<td>0.84 ± 0.06</td>
<td>0.85 ± 0.07</td>
<td>0.81 ± 0.06</td>
<td>0.86 ± 0.05</td>
<td>0.82 ± 0.08</td>
<td>0.83 ± 0.06</td>
</tr>
<tr>
<td>Urine flow, mL · min⁻¹ · g⁻¹</td>
<td>18.3 ± 4.6</td>
<td>13.1 ± 4.2</td>
<td>6.2 ± 2.5</td>
<td>6.5 ± 1.4*</td>
<td>8.1 ± 2.4</td>
<td>6.0 ± 2.5</td>
</tr>
<tr>
<td>Fractional excretion of sodium, %</td>
<td>2.7 ± 0.6</td>
<td>1.8 ± 0.6</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.2*</td>
<td>0.8 ± 0.3</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Urinary potassium excretion, μmol · min⁻¹ · g⁻¹</td>
<td>0.51 ± 0.05</td>
<td>0.48 ± 0.04</td>
<td>0.33 ± 0.05</td>
<td>0.37 ± 0.05*</td>
<td>0.36 ± 0.06</td>
<td>0.30 ± 0.05</td>
</tr>
</tbody>
</table>

Responses were tested in 6 dogs.

*P<0.05 vs control value before NLA.

Effect of NLA Administration on the Medullary NO Responses to Reductions in RAP

Inhibition of NO synthesis by NLA administration resulted in significant reductions in output current and medullary tissue NO concentration (Figure 2). In 1 dog, the recording of NO current during NLA infusion was interrupted by a technical fault. In the other 5 dogs, the mean decrease in medullary NO activity was 218 ± 0.06 nmol · L⁻¹ · min⁻¹ · g⁻¹ (n=5; P<0.05). NO synthase inhibition resulted in significant reductions in U_{NOx,V} (0.8±0.06 nmol · min⁻¹ · g⁻¹) and U_{NOx,V} (1.1±0.2 μmol · min⁻¹ · g⁻¹) (Figure 3). Usual increases in renal vascular resistance and decreases in total and regional renal blood flow, urine flow, and potassium excretion were found during NLA administration, without changes to the GFR (Table).

Stepwise reductions in RAP during NLA administration resulted in attenuation of RAP-dependent changes in medullary NO activity (Figure 2). The mean slope of the relationship between RAP and medullary NO activity was reduced from 10.2±4.5 to 2.7±2.2 nmol · L⁻¹ · mm Hg⁻¹ (P<0.05) during the NLA infusion period. The slopes of the relationship between RAP and U_{NOx,V} and RAP versus U_{NOx,V} were also significantly reduced from 0.02±0.004 to 0.007±0.001 nmol · min⁻¹ · g⁻¹ · mm Hg⁻¹ (P<0.05) and from 0.06±0.02 to 0.01±0.002 μmol · min⁻¹ · g⁻¹ · mm Hg⁻¹ (P<0.05), respectively, during NLA administration (Figure 3). Total and regional blood flows and the GFR remained autoregulated during the NLA infusion period (Table).

Discussion

The technique of using an NO-selective electrode to evaluate renal tissue NO activity in vivo in dogs was validated in our previous study. The generation of output current from the electrode inserted into the renal tissue was shown to be responsive to intra-arterial bolus injections of NO agonists acetylcholine and bradykinin, the NO donor compound SNAP, and the NO synthase inhibitor NLA. Those findings demonstrated the capability of this electrode to determine adequately the dynamic changes in intrarenal NO activity in vivo. In the present study, we observed that acute alterations in RAP within autoregulatory range resulted in linear changes in tissue NO activity in the renal medulla that were associated with decreases in U_{NOx,V} and U_{NOx,V} without changes in total or regional blood flows or GFR. NO synthesis inhibition resulted in reductions in medullary tissue NO activity and attenuated the slope of the relationship between RAP and tissue NO concentrations. These observations further demonstrate the dependence of intrarenal NO activity on renal perfusion pressure and are consistent with the hypothesis that the increases in NO release may be an important determining factor in medullary NO activity.
factor in the sodium excretory responses to acute elevations in RAP.

The exact site and mechanism of changes in NO production rate in response to acute alterations in RAP that contributed to the changes in medullary NO activity are not yet clearly understood. However, studies have suggested that changes in shear stress on the vessel wall during autoregulatory adjustments in preglomerular arteriolar resistance alter endothelial NO release. Although such an alteration in endogenous NO release could predominantly affect the tissue NO activity in the cortex, as it is the segmental region harboring preglomerular vessels, the prevailing tissue level of NO in the renal medulla would be equally affected because of the high diffusive nature of NO in the biological tissue. The results of this investigation and those of our previous study demonstrate that both cortical and medullary tissue NO levels are equally affected by the changes in RAP. However, because the presence of NO synthase enzyme seems to be more in the renal medulla than in the cortex, local generation of NO is likely to have had considerably influenced the changes in medullary tissue NO concentration. This possibility is difficult to explain, because shear stress–mediated changes in endothelial NO release during changes in arterial pressure presumably occur in the preglomerular vessels. How medullary NO synthase could play a role in linking the hemodynamic events with the altered tubular transport response in pressure natriuresis phenomenon is unclear, especially when blood flow to the renal medulla in dogs does not change during alterations in RAP within autoregulatory range.

As expected, medullary NO activity decreased during inhibition of NO synthase by NLA administration in the present study. However, it was noted that the magnitude of the reductions in medullary NO activity (mean, \(-218 \pm 55 \text{ nmol} \cdot \text{L}^{-1}\)) was comparatively much less than what was observed previously in cortical NO activity (mean, \(-573 \pm 127 \text{ nmol} \cdot \text{L}^{-1}\)). The exact reason for this difference in the regional responses to administration of NLA is not yet clear. However, as reported in an earlier study in rats in which the NO-selective electrode was used, this could be related to the augmentation of NO bioavailability in the renal medulla as a result of reduction in tissue oxygen tension during NLA administration. Although oxygen per se does not affect the electrode current, tissue oxygen concentration exerts a critical influence on the NO measurement, because oxygen radicals scavenge NO in the tissue and modulate its bioavailability. Such enhancement of NO bioavailability in the renal medulla may have a critical implication in the regulation of renal salt and electrolyte excretion in the conditions of untoward reduction in tissue oxygen tension, because NO is considered to be an important regulator of deep nephron tubular reabsorptive function.

Although substantial evidence now exists that shows that intrarenal NO may be involved in the mediation of pressure natriuresis, the exact link between RAP-induced changes in NO release and changes in tubular sodium reabsorption remains to be established. As demonstrated in this investigation and our previous study, both medullary and cortical tissue NO activities are altered during acute changes in RAP; it is conceivable that such alterations in NO activity could directly influence the tubular reabsorptive function to cause pressure-induced natriuretic responses in the kidney. Thus, increases in medullary NO activity in response to acute elevations in RAP can be attributed to the inhibition of sodium-reabsorptive function in the deeper nephrons, which are suggested to be primarily involved in the pressure-natriuretic phenomenon. 

RAP-induced alterations in intrarenal NO activity have no significant effect on the total or regional blood flow to the kidney. As explained earlier, the possible action of increased NO activity to cause renal vasodilation during elevations in RAP could be counteracted by the ability of the kidney to exert autoregulatory adjustment in renal vascular resistance, which is essentially autonomous from NO activity. However, such RAP-induced changes in NO activity may affect other hemodynamic changes in the kidney, such as renal interstitial hydrostatic pressure (RIHP), which may exert some effects on tubular reabsorptive function. Mattson et al have shown that inhibition of NO synthase in the renal medulla of rats can cause a decrease in RIHP. We have also observed that RAP-induced changes in RIHP in dogs remained attenuated during NO synthesis inhibition or during constant rate infusion of NO donor compounds, which suggests that changes in intrarenal NO activity are critically linked to changes in RIHP. Such NO-mediated changes in RIHP could also affect the tubular reabsorptive function in response to alterations in RAP.

In conclusion, the results of this study further support the hypothesis that acute changes in arterial pressure result in alterations in intrarenal NO activity, which may directly alter tubular reabsorption rate to manifest the phenomenon of pressure natriuresis.

Acknowledgments

This study was supported by grants from the Louisiana Education Quality Support Fund (LEQSF) and the National Heart, Lung, and Blood Institute, National Institutes of Health (HL-51306). We are grateful to K. Abu Taher and Akira Nishiyama for technical assistance and to Agnes C. Buffone for preparing the manuscript. We are also grateful to Prof L. Gabriel Navar for his valuable suggestions and comments related to this study.

References


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Hypertension. 1999;34:832-836
doi: 10.1161/01.HYP.34.4.832

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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