Intrarenal Nitric Oxide Activity and Pressure Natriuresis in Anesthetized Dogs

Dewan S.A. Majid, Sophia A. Omoro, So Yeon Chin, L. Gabriel Navar

Abstract—Recent studies have indicated that changes in intrarenal nitric oxide (NO) production participate in mediating arterial pressure–induced changes in urinary sodium excretion. Until recently, however, the means to measure changes in intrarenal NO activity in vivo have not been available. For the present study, changes in renal tissue NO activities were assessed directly using an NO-selective microelectrode inserted into the cortical tissue of anesthetized dogs. Control studies demonstrated that the electrode was responsive to intrarenal bolus injections of acetylcholine and to the NO donor S-nitroso-acetylpenicillamine (SNAP). Intrarenal nitro-l-arginine (50 µg · kg⁻¹ · min⁻¹) decreased renal tissue NO concentration by 593 ± 127 nmol/L (P<0.05; n=7). Infusions of SNAP (1, 2, and 3 µg · kg⁻¹ · min⁻¹ for 25 minutes) in nitro-l-arginine–treated dogs (n=5) resulted in dose-dependent increases in renal tissue NO activity, which showed a positive correlation with changes in urinary excretion rates of NO metabolites, nitrates and nitrites, (r=0.62, P<0.05) and sodium (r=0.78, P<0.01). During graded reductions of renal arterial pressure within the autoregulatory range (144±3 to 73±2 mm Hg; n=10), there were decreases in tissue NO activity that were positively correlated with changes in renal arterial pressure (r=0.45; P<0.05), urinary nitrate/nitrite excretion (r=0.64, P<0.005), and urinary sodium excretion (r=0.46; P<0.05). These data support the hypothesis that acute changes in renal arterial pressure result in alterations in intrarenal NO activity, which may be responsible for the associated changes in sodium excretion. (Hypertension. 1998;32:266-272.)

Key Words: nitric oxide electrode • sodium excretion • nitrate excretion • pressure-diuresis

Nitric oxide is known to play a substantive role in the regulation of renal hemodynamics and renal excretory function. Previous studies have suggested that renal production of NO is important in the acute and long-term regulation of sodium and water excretion. In particular, the changes in urinary sodium excretion in response to acute alterations in RAP appear to be closely associated with changes in the intrarenal production rate of NO. In support of this hypothesis, previous studies have demonstrated a positive relationship between RAP and urinary excretion rate of NO metabolites, nitrates and nitrites (NO₂⁻/NO₃⁻), in anesthetized dogs. However, direct in vivo assessment of changes in intrarenal NO activity during changes in RAP has not yet been performed. To understand further the role of NO in the pressure-natriuresis phenomenon, it is important to determine the dynamic changes in intrarenal NO activity during acute changes in RAP. However, the continuous in vivo assessment of intrarenal NO activity has not been possible until recently.

NO-sensing microelectrodes that provide real-time monitoring of NO concentration in biological tissues during both in vitro and in vivo conditions have been developed recently. Among the available types, the platinum/iridium alloy electrode coated with an NO-selective nitrocellulose polymer membrane has been shown to have high sensitivity and applicability to in vivo preparations. The electrode is a polarographic electrode measuring electrochemical oxidation of NO at the electrode surface. In the present study, we evaluated the NO-selective electrode in an effort to obtain a continuous and direct assessment of changes in renal tissue NO activity in vivo, and we examined the effects of acute changes in RAP on cortical NO activity in anesthetized dogs.

Methods

Experiments were performed on 24 mongrel dogs (16 to 23 kg body weight) that were given supplemental amounts of sodium chloride (1.5 gm/kg body wt per day for 3 days) added to the normal laboratory diet so that they achieved a sodium-replete state. Anesthesia was induced in these dogs with sodium pentobarbital (30 mg/kg body wt) and maintained throughout the experiments with additional doses as needed. A cuffed endotracheal tube was inserted into the trachea and connected to an artificial respirator, which was set at a rate of 18 strokes per minute with a stroke volume of 15 mL/kg body wt. Body temperature was maintained at a near constant level (≈100°F) with an electric heating pad. SAP was measured from a catheter placed in the abdominal aorta inserted through the right femoral artery. The catheter was connected to a pressure transducer, and SAP 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 blood samples. The femoral and jugular veins were cannulated for

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The left kidney was exposed through a flank incision, and the renal artery was isolated from surrounding tissue. Renal denervation was performed by cutting all the visible nerves projecting to the kidney from the aortico-adrenal ganglion. RBF was measured with an EMF placed on the renal artery near its origin from the aorta and connected to a square wave flowmeter (Carolina Medical Electronics). A curved 23-gauge needle cannula was inserted into the renal artery distal to the flow probe and was connected to a pressure transducer for measurement of RAP. Another catheter was also connected to this needle cannula for continuous infusion of heparinized saline or drug solutions at a rate of 0.4 mL/min. In the dogs in which responses to acute reductions in RAP were examined, an adjustable plastic clamp was placed on the renal artery between the flow probe and the needle cannula. In some dogs, the relative changes in regional blood flow were measured using 2 needle probes connected to a dual-channel laser-Doppler flowmeter (Periflux 4001, Perimed) as described previously.19 The tips of these needle probes were inserted into the kidney to depths of 5 mm and 15 mm to measure renal cortical and medullary blood flow responses, respectively. 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 recalibrated with a standard calibration device using a motility standard as described previously.17

To measure the dynamic changes in renal tissue NO activities, an NO-selective microelectrode (Inter Medical Co) was inserted into the renal cortex. This system consists of a working electrode and a reference electrode. The electrode is made of platinum/iridium alloy and is 200 μm in diameter. The electrode is small and sufficiently sturdy so that it can be inserted directly into the tissue. The tip of the electrode is coated with 3 layers consisting of KCl, an NO-selective membrane, and a normal silicon membrane. The KCl membrane is used to suppress overvoltage in the NO discharge. The NO-selective membrane is made of nitrocellulose, which is permeable to only small gaseous molecules. The silicon membrane is affixed to avoid a nonspecific ionic effect and electrochemical reactions. The electrode is a polargraphic electrode measuring electrochemical oxidation of NO at the electrode surface (NO+4OH →NO2−+2H2O+3E−). NO activity is based on the current induced by the electrochemical reaction. The resulting polarographic current is detected with a current voltage converter circuit used in the NO monitor. The reference electrode is made of carbon fibers.

Each NO electrode was calibrated in vitro by adding known doses of the NO donor compound SNAP to a cuvette in which the electrode was immersed. Figure 1 illustrates the average calibration curve generated from various electrodes showing that the current generated in the electrode is linearly related to the dose of SNAP. The estimated NO concentrations provided by the SNAP doses are also depicted in this figure. Although the SNAP doses show a linear dose-response relationship with output current from individual electrodes, there was some variation among electrodes as reflected by the wide standard deviation of the mean current values generated by the SNAP concentrations. To measure renal tissue NO concentration, the NO electrode was inserted 5 mm into the cortex after a small portion of the renal capsule was removed. The reference electrode was placed on the surface of the kidney underneath the capsule. The NO electrode in the renal cortex was found to be responsive to intra-arterial bolus injections of NO agonists acetylcholine and bradykinin, the NO donor SNAP, and the NO synthase inhibitor NLA. An example of the response to acetylcholine is shown in Figure 2. Equivolume injection of saline vehicle (1 mL) in the renal artery did not cause any change in output currents from the electrode (not shown in Figure 2). Because of variation in baseline currents from electrodes inserted in tissues, the absolute signals do not reflect the basal level of NO activity in the renal tissue. However, the data provide an index of changes in tissue NO concentration based on the changes in output current and on the in vitro calibrations. Therefore, only changes in output currents and in tissue NO concentrations are considered in this study. The stability of the electrodes was evaluated by monitoring outputs for periods of >1 hour in the absence of any experimental manipulations. It was observed that output currents reached a steady state within 15 to 20 minutes of introduction of the electrode in the renal cortex. The mean output current was 9635±977 pA (n=18) after a steady state was reached and was not altered significantly during the period of stabilization, which remained at 9607±992 pA. The simultaneous use of the EMF probe did not influence the NO electrode current, since no changes in output currents were observed when the EMF probe was turned off at the time of the use of the NO electrode.

To minimize respiratory movement artifacts in NO-electrode output signal recordings, as well as in the laser-Doppler flowmeter signals, the kidney was kept in a fixed position by placing it on a plastic holder similar to that used for micropuncture experiments. Care was taken to avoid any reductions in basal RBF after immobilization of the kidney. Urine was collected from a catheter inserted

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**Selected Abbreviations and Acronyms**

- **EMF**: electromagnetic flow probe
- **NLA**: nitro-L-arginine
- **NO**: nitric oxide
- **RAP**: renal arterial pressure
- **RBF**: renal blood flow
- **RIHP**: renal interstitial hydrostatic pressure
- **SAP**: systemic arterial pressure
- **SNAP**: S-nitroso-N-acetylpenicillamine

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**Figure 1.** In vitro calibration curve of the NO electrode (n=6). Doses of the NO donor SNAP were used for calibration. Equivalent NO concentrations of SNAP doses are also depicted on the x-axis.

**Figure 2.** Example of the responses to intra-arterial bolus doses of acetylcholine (Ach) on NO electrode current. Equivolume injection of saline vehicle (1 mL) did not cause any change in output currents from the NO electrode (not shown). Deflections in RAP traces are the injection artifacts.
into the ureter. After completion of surgical procedures, at least 1 hour was allowed for stabilization before the start of the experimental protocol. In the dogs in which urine collections were made, a 2.5% solution of inulin in normal saline was administered into the jugular vein for at least 45 minutes before urine collections. An initial dose of 1.6 mL · kg⁻¹ was followed by a continuous infusion of 0.03 mL · kg⁻¹ · min⁻¹.

The applicability of the use of this NO-selective electrode was examined in 14 dogs. Bolus doses of SNAP (0.25, 0.5, 2.5, and 5 µg · kg⁻¹) were administered intra-arterially in 7 dogs to examine the effects of increases in intrarenal NO activity. Decreases in renal tissue NO activity were demonstrated by the administration of the NO synthase inhibitor NLA at 50 µg · kg⁻¹ · min⁻¹ for 30 minutes in another 7 dogs. To examine the relationship between the changes in tissue NO concentration and the changes in urinary excretion of the NO metabolites NO₂⁻/NO₃⁻, continuous infusions of SNAP at 1, 2, and 3 µg · kg⁻¹ · min⁻¹ were administered in 5 dogs pretreated with NLA. After initiation of the SNAP infusions, 5 minutes were allowed for stabilization before two 10-minute collections of urine with midpoint collections of arterial blood samples (2 mL) were taken. It was noted that such blood sampling did not affect the electrode currents.

The effects of changes in RAP on renal tissue NO activity were examined in 10 dogs. In these dogs, the right common carotid artery was occluded and the left common carotid artery was partially constricted to elicit a baroreflex and elevate basal arterial pressure to approximately 150 mm Hg at least 45 minutes before the start of the experimental protocol. This partial carotid occlusion was maintained throughout the experimental period. Urine samples for 2 consecutive 10-minute periods were collected at spontaneous RAP. An arterial blood sample (2 mL) was collected at the midpoint of each urine collection period. RAP was then reduced in steps of approximately 25 mm Hg by adjustment of the arterial occluder. Five minutes were allowed for stabilization at each level of RAP before a 10-minute urine sample was collected. After the last reduction in RAP, the occluder was released completely to reestablish control RAP and RBF.

At the end of each experiment, the EMF probe was calibrated in situ by collection of timed blood samples into a graduated cylinder at different flows from a catheter placed in the renal artery. The kidney was then removed, stripped of all surrounding tissue, blotted dry, and weighed so that the calculated parameters could be expressed per gram of kidney tissue. Flame photometry (Instrumentation Laboratory) was used to determine the sodium and potassium concentrations in plasma and urine. Inulin concentrations in plasma and urine samples were determined by the anthrone colorimetric technique (Gilford Instruments). Urinary concentrations of NO₂⁻/NO₃⁻ were measured using the Greiss reaction technique after enzymatic reductions of nitrate to nitrite in the samples as described previously.

Values are reported as mean±SEM. Statistical comparisons of differences in the responses were conducted with the use of ANOVA followed by Newman-Keuls test. Differences in the mean values were deemed significant at P≤0.05. Correlation of the responses were made by Pearson product moment correlation analysis using the Sigmastat statistical program.

Results

Effects of Bolus Administration of SNAP on Renal Tissue NO Activity

Bolus injections of SNAP at doses of 0.25, 0.5, 2.5, and 5 µg · kg⁻¹ intra-arterially resulted in dose-dependent increases in output current from the NO electrode placed in the cortex. Figure 3 illustrates the average results in 7 dogs. Changes in output currents of 125±17, 386±91, 579±109, and 843±133 pA occurred in response to the SNAP infusions, respectively (Figure 3A). On the basis of in vitro calibration of the electrode, changes in tissue NO activity were estimated and are depicted in Figure 3B. Increasing doses of SNAP resulted in increases in tissue NO activity of 80±18, 220±41, 357±82, and 519±114 nmoL/L, respectively.

Effects of NLA Administration on Renal Tissue NO Activity

Intra-arterial administration of NLA at a rate of 50 µg · kg⁻¹ · min⁻¹ for 30 minutes in 7 dogs resulted in consistent decreases in output currents from the NO electrodes. Figure 4A illustrates the average results in these dogs. The mean decrease in tissue NO activity (573±127 nmoL/L, P<0.001) estimated from the in vitro calibrations is depicted in Figure 4B. The associated changes in renal hemodynamics and renal excretory function are shown in Table 1.

Relation Between Tissue NO Activity and Urinary Excretion Rate of NO Metabolites NO₂⁻/NO₃⁻

During continuous infusion of SNAP at 1, 2, and 3 µg · kg⁻¹ · min⁻¹ for 25 minutes in dogs pretreated with NLA (50 µg · kg⁻¹ · min⁻¹, n=5), there were parallel increases in renal tissue NO activity and urinary excretion rate of NO₂⁻/NO₃⁻. Figure 5 illustrates the changes in output current (A) and the estimated changes in tissue NO activity (B) during SNAP infusions in NLA-treated dogs. In 1 of these dogs, the recordings of output currents during the higher 2 doses of SNAP infusions were interrupted because of technical problems. It was noted that at the highest dose of SNAP, there was an attenuation of the responses from the electrode. This may be because of increased NO washout from the tissue due to increases in RBF caused by SNAP. There were dose-dependent increases in RBF, urine flow, sodium excretion, fractional excretion of sodium, urinary excretion of NO₂⁻/NO₃⁻, and decreases in renal vascular resistance without
TABLE 1. Effects of SNAP Infusions in Dogs Treated With NLA (n=5)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NLA Alone</th>
<th>SNAP, 1 µg · kg⁻¹ · min⁻¹</th>
<th>SNAP, 2 µg · kg⁻¹ · min⁻¹</th>
<th>SNAP, 3 µg · kg⁻¹ · min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP, mm Hg</td>
<td>131±7.9</td>
<td>140±7.6*</td>
<td>135±8.9</td>
<td>127±7.3†</td>
<td>123±6.4†</td>
</tr>
<tr>
<td>RVR, mm Hg · mL⁻¹ · min⁻¹ · g⁻¹</td>
<td>32.3±1.7</td>
<td>49.3±1.5*</td>
<td>44.7±3.5†</td>
<td>39.8±3.1†</td>
<td>35.9±2.2†</td>
</tr>
<tr>
<td>GFR, mL · min⁻¹ · g⁻¹</td>
<td>4.12±0.12</td>
<td>2.94±0.19*</td>
<td>3.17±0.21</td>
<td>3.26±0.16†</td>
<td>3.43±0.17†</td>
</tr>
<tr>
<td>Urine flow, µL · min⁻¹ · g⁻¹</td>
<td>8.6±1.7</td>
<td>3.8±1.0*</td>
<td>7.1±2.1†</td>
<td>10.6±2.3†</td>
<td>11.8±1.2†</td>
</tr>
<tr>
<td>UaV, µmol · min⁻¹ · g⁻¹</td>
<td>1.67±0.24</td>
<td>0.51±0.18*</td>
<td>0.94±0.31</td>
<td>1.78±0.36†</td>
<td>2.11±0.30†</td>
</tr>
<tr>
<td>Fractional excretion of sodium, %</td>
<td>1.58±0.21</td>
<td>0.51±0.15*</td>
<td>0.88±0.39</td>
<td>1.55±0.27†</td>
<td>2.10±0.31†</td>
</tr>
<tr>
<td>UNO⁻-NO₂⁻V, nmol · min⁻¹ · g⁻¹</td>
<td>0.92±0.17</td>
<td>0.41±0.07*</td>
<td>0.65±0.12†</td>
<td>0.99±0.15†</td>
<td>1.29±0.18†</td>
</tr>
</tbody>
</table>

RVR indicates renal vascular resistance; GFR, glomerular filtration rate; UaV, urinary sodium excretion; and UNO⁻-NO₂⁻V, urinary nitrate/nitrite excretion.

*P<0.05 vs control; †P<0.05 vs NLA alone.

Discussion

The application of the NO-selective electrode to measure NO activity in vivo has been limited. Because NO decays within seconds, dynamic assessment of changes in tissue NO activity is particularly important. The polymer-coated NO-selective electrode used in this study has been shown to have high sensitivity and selectivity for NO. Oxygen per se does not contribute substantively to the electrode current because the applied voltage on the working electrode from 0.4 to 0.8 V lies in a range where oxygen is not electrolyzed. However, the presence of oxygen radical superoxide (O₂⁻) can reduce the NO concentration in the tissue by its scavenging effect on NO and can indirectly affect the electrode current. The secondary products of NO oxidation, NO₃⁻/NO₂⁻, also have been shown to not interfere with the electrode current. Body temperature was maintained at a relatively constant level (~100°F) in these experiments, thus minimizing possible effects of changes in temperature on the electrode current. The output current from the electrode in the renal cortex also responded dose-dependently to intra-arterial administration of NO agonists (Figure 2), an NO donor (SNAP, Figures 3 and 5), and an NO synthase inhibitor (NLA, Figure 4), indicating that the NO electrode has enough sensitivity to detect biologically generated NO in the kidney. The observation that the concomitant changes in urinary excretion rate changes in glomerular filtration rate (Table 1). The changes in tissue NO concentration during SNAP infusions were positively correlated with changes in urinary excretion of NO₃⁻/NO₂⁻ (r=0.64, P<0.05; Figure 6A) and of sodium (r=0.79, P<0.005; Figure 6B).

Figure 5. Responses to intra-arterial infusion of SNAP in output current from the NO electrode (A) and tissue NO activity (B, calculated from in vitro calibration curve) in dogs pretreated with NLA (50 µg · kg⁻¹ · min⁻¹; n=5). *P<0.05 vs NLA treatment.

Figure 6. Relationship between changes in renal tissue NO activity and changes in urinary nitrate and nitrite excretion (A) as well as sodium excretion (B) during intra-arterial infusion of SNAP doses in 5 dogs. In 1 of these dogs, the recordings of the output currents from the electrode during infusions of 2 higher SNAP doses were interrupted because of technical problems. Thus, there are 13 data points instead of 15 for the number of measurements in these dogs.
of the NO metabolites NO$_3^-$/NO$_2^-$ showed a positive correlation with the changes in tissue NO activity during intra-arterial infusions of SNAP (Figure 6A) also supports the capability of this electrode to monitor intrarenal NO activity in vivo.

The results of the present study provide direct evidence that acute alterations in RAP within the autoregulatory range induce concomitant changes in intrarenal NO activity even in the presence of highly efficient autoregulation of total and regional blood flows. The changes in tissue NO activity in response to acute changes in RAP showed a strong positive correlation with the concomitant changes in urinary excretion rate of the NO metabolites NO$_3^-$/NO$_2^-$ (Figure 9), further supporting the dependency of intrarenal NO activity on renal perfusion pressure. In the presence of an intact autoregulatory system, an acute elevation of RAP results in vasoconstriction in preglomerular resistance vessels, which leads to increases in blood flow velocity in the vessels to maintain the same absolute blood flow. Such changes in arterial blood flow velocity could exert shear stress on the vessel wall and thus induce alterations in the basal release of NO. Because of the high diffusive capability of NO, the prevailing tissue level of NO in the kidney would increase after enhanced production of endothelial NO during acute increases in RAP. Although such increases in NO production would be expected to cause renal vasodilation, RBF did not change during acute increases in RAP. However, it should be emphasized that the results of the present study do not indicate that NO is not important in the control of intrarenal blood flow within the autoregulatory range. Previous studies (References 4, 5, 7, 23, and 33 and review articles 1, 2, and 10) clearly demonstrate that NO exerts a substantive role in regulating renal vascular tone by influencing primarily the autoregulation-independent component of renal vascular resistance. The results from the present as well as previous studies suggest that during changes in RAP, the possible effects of altered NO activity on RBF are counteracted by the ability of the kidney to exert regulatory adjustments in the autoregulation-responsive component of vascular resistance, which is essentially autonomous from NO activity.

In these experiments, we observed a positive correlation between the changes in tissue NO activity and the concomitant changes in sodium excretion during alterations in RAP within the autoregulatory range (Figure 8B). These findings further support the hypothesis that the renal synthesis of NO is an important factor in determining the sodium excretory function.

![Figure 7](image7.png)

**Figure 7.** Example of the changes in NO electrode current observed during alterations in RAP. The NO electrode was inserted into the renal cortex. Step changes in RAP show reductions in NO current, which returned toward the control value after the release of renal artery constriction (RAC). RBF remained autoregulated during reductions in RAP.

![Figure 8](image8.png)

**Figure 8.** Changes in output current from the NO electrode in renal cortex (A) and tissue NO activity (B) during stepwise reductions in RAP within autoregulatory range (n=10). *P<0.05 versus control values at spontaneous RAP (~150 mm Hg).

![Figure 9](image9.png)

**Figure 9.** Relationship between changes in renal tissue NO activity and changes in urinary nitrate and nitrite excretion (A) as well as urinary sodium excretion (B) during changes in RAP in 10 dogs.

### Table 2: Renal Responses to Reductions in RAP in Anesthetized Dogs (n=10)

<table>
<thead>
<tr>
<th>RAP, mm Hg</th>
<th>RVR, mm Hg · mL$^{-1}$ · min$^{-1}$ · g$^{-1}$</th>
<th>RBF, mL · min$^{-1}$ · g$^{-1}$</th>
<th>Cortical blood flow, % of control (n=6)</th>
<th>Medullary blood flow, % of control (n=6)</th>
<th>GFR, mL · min$^{-1}$ · g$^{-1}$</th>
<th>U$_{\text{Na}}$, mmol · min$^{-1}$ · g$^{-1}$</th>
<th>Fractional excretion of sodium, %</th>
<th>U$_{\text{NO}}$, nmol · min$^{-1}$ · g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>144±2.9</td>
<td>40.1±4.0</td>
<td>3.90±0.35</td>
<td>100</td>
<td>100</td>
<td>0.80±0.07</td>
<td>2.76±0.50</td>
<td>2.48±0.44</td>
<td>1.18±0.16</td>
</tr>
<tr>
<td>112±1.7</td>
<td>30.2±2.6*</td>
<td>3.98±0.33</td>
<td>103±2.0</td>
<td>98±2.8</td>
<td>0.78±0.05</td>
<td>1.92±0.33*</td>
<td>1.73±0.33*</td>
<td>0.93±0.14*</td>
</tr>
<tr>
<td>73±2.4</td>
<td>19.1±1.6*†</td>
<td>4.06±0.33</td>
<td>106±4.8</td>
<td>108±7.4</td>
<td>0.77±0.08</td>
<td>0.84±0.21†</td>
<td>0.78±0.20†</td>
<td>0.52±0.14†</td>
</tr>
</tbody>
</table>

Abbreviations are defined in Table 1. *P<0.05 vs values at RAP 144 mm Hg. †P<0.05 vs values at RAP 112 mm Hg.
responses to acute changes in arterial pressure, NO can exert its effects on sodium excretion either by influencing tubular transport directly or by altering the intrarenal hemodynamic environment. Some evidence is available that NO exerts a direct inhibitory effect on epithelial transport mechanisms. In a series of experiments performed in cultured cortical collecting duct cells, as well as in isolated perfused collecting duct segments, Stooß and colleagues demonstrated that the amiloride-sensitive sodium transport pathway is inhibited by NO. It is therefore conceivable that enhancement of renal tissue NO concentration in response to acute increases in RAP, as observed in this study, may directly inhibit tubular sodium transport, thus leading to an increase in sodium excretion. The source of enhanced NO production in the kidney during increases in RAP has not been determined definitively. However, because increases in RAP exert alterations in blood flow velocity and shear stress in the preglomerular vessels, it is reasonable to assume that renal endothelial NO synthase activity in preglomerular arterioles is responsible for enhanced NO production rate. Such an increased release of NO from endothelial cells during elevated RAP could diffuse throughout the parenchyma to exert its inhibitory effects on sodium transport. This assumption is supported by the fact that there are abundant close contacts between afferent arterioles and distal tubules, which suggests a relatively short diffusion pathway from preglomerular vessels to distal nephron segments.

It has been suggested that the deeper nephrons rather than the superficial cortical nephrons are primarily involved in the pressure-natriuretic phenomenon; therefore, it is possible that medullary rather than cortical tissue NO activity is of more direct interest in relation to the present study. For this initial study, however, emphasis was placed on cortical NO activity because the cortex is the principal site where tissue NO activity would be affected by the shear stress-induced changes in NO generation in the preglomerular vessels in response to changes in RAP. After alterations in NO generation during changes in RAP, the prevailing tissue concentrations of NO might be equally affected both in cortex and in medulla because of the high diffusive capability of NO. It is also possible that NO in the medullary tissue may be biologically more active than the NO in cortical tissue due to the difference in the tissue oxygen tension in these 2 regions. Thus, changes in NO activity during changes in RAP may affect reabsorptive function comparatively more in the deeper nephrons than in the superficial nephrons. Future studies to measure medullary NO concentrations may be needed to explain more comprehensively this issue of NO-dependent changes in tubular sodium reabsorption.

It has also been reported that neuronal NO synthase is located in macula densa cells and other regions in the kidney. NO produced by macula densa cells has been postulated to play a role in tubuloglomerular feedback responses. Thus, it may be possible that increases in RAP cause an enhancement of NO release from the macula densa that may travel downstream in the tubular fluid to the distal nephron segments to cause inhibition of sodium transport. NO can also be formed in collecting duct cells and affect sodium reabsorption rate directly. However, it is not apparent how NO derived from epithelial cells could participate in the responses to increases in RAP, since this would not explain the critical link between the hemodynamic events in preglomerular vessels and increased intrarenal levels of NO. It is also possible that increases in intrarenal NO activity during increases in RAP influence tubular transport by altering the intrarenal hemodynamic environment, such as RHP. It has been reported that RHP can be reduced by selective inhibition of NO in the rat renal medulla and can be increased by the administration of L-arginine in Dahl salt-sensitive rats. These data suggest that changes in intrarenal NO during alterations in RAP may induce changes in RHP. However, further experiments are needed to examine the possible link between RHP and intrarenal NO response to changes in RAP.

In conclusion, the results of the present study support the applicability of the NO electrode for in vivo assessment of intrarenal NO activity. These data are consistent with the hypothesis that acute changes in RAP elicit parallel changes in intrarenal NO activity that alter sodium excretion rate to manifest the phenomenon of pressure natriuresis.

Acknowledgments

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