Role of Nitric Oxide in the Control of Renal Oxygen Consumption and the Regulation of Chemical Work in the Kidney

Sarra K. Laycock, Traci Vogel, Paul R. Forfia, Joshua Tuzman, Xiaobin Xu, Manuel Ochoa, Carl I. Thompson, Alberto Nasjletti, Thomas H. Hintze

Abstract—Inhibition of NO synthesis has recently been shown to increase oxygen extraction in vivo, and NO has been proposed to play a significant role in the regulation of oxygen consumption by both skeletal and cardiac muscle in vivo and in vitro. It was our aim to determine whether NO also has such a role in the kidney, a tissue with a relatively low basal oxygen extraction. In chronically instrumented conscious dogs, administration of an inhibitor of NO synthase, nitro-L-arginine (NLA, 30 mg/kg IV), caused a maintained increase in mean arterial pressure and renal vascular resistance and a decrease in heart rate (all \( P<0.05 \)). At 60 minutes, urine flow rate and glomerular flow rate decreased by 44±12% and 45±7%, respectively; moreover, the amount of sodium reabsorbed fell from 16±1.7 to 8.5±1.1 mmol/min (all \( P<0.05 \)). At this time, oxygen uptake and extraction increased markedly by 115±37% and 102±34%, respectively (\( P<0.05 \)). Oxygen consumption also significantly increased from 4.5±0.6 to 7.1±0.9 mL O\(_2\)/min. Most important, the ratio of oxygen consumption to sodium reabsorbed increased dramatically from 0.33±0.07 to 0.75±0.11 mL O\(_2\)/mmol Na\(^+\) (\( P<0.05 \)), suggesting a reduction in renal efficiency for transporting sodium. In vitro, both a NO-donating agent and the NO synthase–stimulating agonist bradykinin significantly decreased both cortical and medullary renal oxygen consumption. In conclusion, NO plays a role in maintaining a balance between oxygen consumption and sodium reabsorption, the major ATP-consuming process in the kidney, in conscious dogs, and NO can inhibit mitochondrial oxygen consumption in canine renal slices in vitro. (Circ Res. 1998;82:1263-1271.)

Key Words: nitro-L-arginine ■ Na\(^+\) reabsorption ■ nitrate/nitrite ■ renal slice

The endogenously and tonically produced vasodilator NO has been implicated in the control of oxygen consumption. Evidence of such a role for NO was initially provided by Granger and Lehninger\(^1\) when they observed an inhibition of mitochondrial electron transport, indicated by a decrease in oxygen consumption, in a murine cell line exposed to endotoxin-stimulated macrophages. NO was subsequently suggested to be the mediator of this phenomenon,\(^2\) and these observations have sparked a number of studies investigating this novel role for NO. These studies have demonstrated that NO has a direct and reversible inhibitory action on cytochrome \( c \) oxidase in rat mitochondria,\(^3,4\) perhaps via binding to the heme of cytochrome \( a_\alpha \). Moreover, administration of NO has also been observed to inhibit complexes I and II,\(^5\) an effect thought to be mediated by peroxynitrite, the product of the reaction of NO with superoxide.\(^5\)

NO-donating drugs have also been demonstrated to inhibit the oxygen consumption of skeletal and cardiac muscle in vitro.\(^7,8\) In addition, oxygen consumption was also reduced in response to agonists known to stimulate the production of NO, an effect that was completely attenuated by pretreatment with a NOS inhibitor. Indeed, NOS inhibitors have been demonstrated to increase the oxygen consumption of the whole body as well as the skeletal muscle and heart of conscious dogs,\(^9,10\) suggesting a basal modulatory role for NO on mitochondrial function. In our studies using skeletal and cardiac muscle, the relationship between the mechanical work performed by these muscles and oxygen consumption was shifted after NOS inhibition, indicating that NO may help to maintain mechanical efficiency. However, these studies have been criticized, since it is difficult to determine the relationship between mechanical work and cellular ATP utilization in muscle in vivo. In contrast, the main ATP-consuming process in the kidney is operation of the sodium/potassium ATPase to reabsorb sodium from the glomerular filtrate. This measure of chemical work performed by the kidney has been shown to be directly proportional to oxygen consumption.\(^12\) Moreover, because of the relatively high blood flow and a low extraction of oxygen, the kidney can increase its oxygen consumption greatly. Thus, the aim of the present study was 3-fold: (1) to

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examine the effect of NOS inhibition on the oxygen consumption of the kidney in conscious dogs, (2) to correlate this with the main energy-consuming process in this organ, sodium reabsorption, and (3) to assess whether exogenously or endogenously applied NO could alter renal oxygen consumption in vitro.

Materials and Methods

**Surgical Preparation**

Ten female hound dogs (23 to 30 kg) were sedated with 10 mg IM acepromazine maleate (Fernta Animal Health Co) and anesthetized with 25 mg/kg sodium pentobarbital IV (Nembutal, Abbott Laboratories). An endotracheal tube was then inserted, and the animal was artificially ventilated with room air using a Harvard respirator (Harvard Apparatus). Under sterile conditions, a midline abdominal incision was made, and a pulsed Doppler flow probe (internal diameter, 5 to 6 mm; Harvard Medical School) was placed on the left renal artery proximal to its bifurcation. Tygon catheters (Cardiovascular Instrument Corp) were placed into the abdominal aorta and into the left renal vein via the ovarian vein. The wires and catheters were tunneled through the abdominal wall and under the skin and exteriorized between the scapulae. The abdomen was closed in layers, and antibiotics were administered for 7 days after surgery (400 mg/d IM amoxicillin, Amoxi-inject, SmithKline Beecham Animal Health). After the animals had recovered fully, they were trained to lie quietly on a laboratory table.

All the procedures were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (publication 93–23, revised 1985).

**Measurements of Hemodynamics and Renal Function**

On the day of study, the left renal blood flow probe was connected to a pulsed Doppler flowmeter (Triton) to measure renal blood flow, and the abdominal aortic catheter was connected to a pressure transducer (P23XL, Spectramed) and a preamplifier to measure arterial blood pressure. Both of these parameters were measured continuously throughout the experimental protocol on a chart recorder (model 2800S, Gould) after amplification by a DC amplifier (Gould). Mean arterial blood pressure and mean renal blood flow were derived using a 2-Hz low-pass filter. Under local anesthesia with 2% lidocaine HCl gel (Xylocaine, Astra), a 12F Foley catheter (American Pharmaseal Co) was inserted into the bladder via the urethra. Through this catheter, urine was collected over 20-minute periods, and average UV was calculated for each collection period. A catheter was inserted percutaneously into a peripheral leg vein for the administration of drugs.

**Experimental Protocol**

After baseline hemodynamics had stabilized, two successive 20-minute control urine collection periods were performed to ensure that stable baseline UV had been attained. Before the end of each urine collection period, arterial and renal venous blood samples (7 mL) were simultaneously withdrawn into heparinized syringes, capped, and placed on ice. Confirmation that the renal venous catheter had remained in the renal vein after surgery was achieved by comparison of the renal venous PO2 with that of a venous blood sample concurrently withdrawn from a peripheral leg vein. The PO2 values of the renal and peripheral venous blood samples were 55±1 and 43±3 mm Hg, respectively (P<0.05).

Immediately after the end of the second control urine collection period, 30 mg/kg of NLA (Aldrich Chem Co) dissolved in saline to a concentration of 7 mg/mL was administered intravenously over 3 minutes. The animal was then studied for a further 80 minutes, ie, 4 additional urine collection periods after NLA administration. Urine samples were placed on ice immediately after collection and frozen for later analysis.

**Analysis of Blood Samples**

The total oxygen content of each blood sample was determined on the day of the experiment as described by us previously. Briefly, the amount of oxygen dissolved in the blood was calculated as 0.003×P02, and the PO2, PCO2, and pH values were determined using a Blood Gas Analyser (model 170, Corning Medical). The amount of oxygen bound to hemoglobin, the oxygen content, was determined using a Co-Oximeter (model IL 482, Instruments Laboratory). Thus, the total oxygen content of each blood sample was equal to (0.003×P02)+oxygen content. The oxygen uptake by the kidney was calculated as the arterial total oxygen content minus the renal venous total oxygen content. Oxygen extraction was calculated as the oxygen uptake as a percentage of the arterial total oxygen content; oxygen consumption by the kidney was calculated as oxygen uptake multiplied by renal blood flow at the time of blood sample withdrawal. Hematocrits were determined from the arterial blood samples by centrifugation. Plasma was obtained from the blood samples by centrifugation at 3000 rpm for 15 minutes at 4°C and frozen for later analysis.

**GFR and Ionic Composition**

Creatinine clearance was used to estimate GFR. The creatinine content of the arterial plasma and urine samples was assessed spectrophotometrically using a commercially available kit (Creatinine Kit 555-A, Sigma Chemical Co). The sodium, potassium, and chloride content of the arterial plasma and urine samples were determined electrochemically (model 644 Na/K/Cl Analyser, Ciba-Corning Diagnostics Corp).

**Determination of NOx**

The concentration of NO metabolites, ie, NOx, in arterial plasma and urine samples was determined using a chemiluminescence method described by us previously. Briefly, the nitrate and nitrite in the sample was converted to the gaseous NO by incubation with Aspergillus nitrate reductase, followed by acidification under an argon atmosphere. The amount of NO in the head space gas was then quantified by injection into a NO chemiluminescence analyzer (Sievers Inc) and quantified by comparison with a standard curve. The standard curve was generated each day by dissolving known amounts of sodium nitrite or sodium nitrate (Sigma) in either plasma pooled from sham-operated dogs or control urine collected from each individual animal.

**Effect of NO on Renal Oxygen Consumption In Vitro**

The oxygen consumption of renal tissue slices was measured in vitro polarographically using a method described by us previously. Briefly, left kidneys were obtained from normal male dogs (n=6) under sodium pentobarbital anesthesia (25 mg/kg IV) and placed in ice-cold HEPES buffer. The kidney was immediately decapsulated, and slices of medulla and cortex, ~1 mm thick and weighing between 30 and 50 mg, were prepared. After incubation in Krebs buffer bubbled with 5% CO2/95% air at 37°C for 2 hours, the oxygen consumed by each slice in 3 mL of air-saturated HEPES buffer was measured using a Clarke-Type oxygen electrode (model 5331, YSI Co Inc) connected to a biological oxygen monitor (model 5300, YSI Co Inc) on a linear chart recorder (model 1202, Barnstead/Ther-
molyne Corp). Cumulative dose-response curves to either the NO-donating agent SNAP (10⁻³ to 10⁻⁴ mol/L) or the NOS-activating agonist bradykinin (10⁻⁷ to 10⁻⁵ mol/L) were then constructed in the presence or absence of the NOS inhibitor L-NAME (10⁻⁷ mol/L). Sodium cyanide (10⁻⁴ mol/L) was administered at the end of each dose-response curve to confirm that the oxygen consumption was mitochondrial. Both buffers contained the following (mmol/L): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1, CaCl₂ 5, and glucose 5. In addition, Krebs buffer contained 25 mmol/L NaHCO₃ and HEPES buffer contained 10 mmol/L HEPES and was set at a pH of 7.4 using NaOH. All drugs and chemicals were obtained from Sigma unless otherwise stated.

### Statistical Analysis of Data

All data are expressed as mean±SEM. Statistical analysis was carried out using a one-way ANOVA for repeated measures followed by a Dunnett post hoc test and a 2-way ANOVA for repeated measures followed by an F test or a paired Student t test where appropriate. A value of P<0.05 was considered statistically significant. Commercially available software was used for statistical analysis and graphics (Sigma-Stat version 1.0, Jandel Corp, and SlideWrite version 3.0, Advanced Graphics Software Inc, respectively).

### Results

Although hemodynamic data were collected continuously, and urine and blood samples were collected every 20 minutes, only the data at the 20- and 60-minute time points will be presented in detail in the text. All of the data collected will be presented in the figures.

**Hemodynamic Effects of NOS Inhibition**

Administration of NLA (30 mg/kg) produced significant increases in systolic, diastolic, and mean arterial pressures, which were maximal by 30 minutes (Table 1). Systolic and diastolic arterial pressures at this time point were 35±11% and 44±13% higher than control values. Heart rate decreased significantly, decreasing by 31±8% from control at 30 minutes. Both the increase in mean arterial blood pressure and the decrease in heart rate were maintained for the duration of the experiment.

Mean renal blood flow decreased by 22±6% at 20 minutes after NLA administration; however, this significant decrease was only transient returning to control values by 40 minutes (Figure 1). Renal vascular resistance increased significantly by 10 minutes after the administration of NLA, reaching a maximum of 76±23% greater than control values by 30 minutes, and remained significantly elevated for the remainder of the experiment (Figure 1).

**Effects of NOS Inhibition on Renal Function**

Both GFR and UV decreased significantly in response to NOS inhibition (Table 2). At 60 minutes after NLA administration, the respective GFR and UV values were 45±7% and 44±12% lower than control values (both P<0.05). Sodium excretion was decreased from 49±9 to 9±3 μmol/min at 60 minutes after the administration of NLA (P<0.05).

Sodium was being actively conserved by the kidneys in these animals, as evidenced by the high fractional reabsorption in the control state (Table 2). This almost maximal reabsorption of sodium was unaltered by the administration of NLA; however, as a result of the decrease in GFR, the filtered load of sodium decreased significantly from the control value. Hence, despite a maintained fractional reabsorption of sodium, the amount of sodium reabsorbed fell by 44±7% at 60 minutes after NLA (P<0.05) (Table 2).

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**Table 1. Effects of NOS Inhibition, With NLA, on Hemodynamics**

<table>
<thead>
<tr>
<th>Time After NLA, min</th>
<th>Control</th>
<th>20</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>110±3</td>
<td>133±4*</td>
<td>142±4*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>80±4</td>
<td>47±2*</td>
<td>56±4*</td>
</tr>
<tr>
<td>mRBF, mL/min</td>
<td>259±20</td>
<td>197±19*</td>
<td>248±27</td>
</tr>
<tr>
<td>RVR, mm Hg · mL⁻¹ · min⁻¹</td>
<td>0.229±0.016</td>
<td>0.343±0.023*</td>
<td>0.330±0.022*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; HR, heart rate; mRBF, mean renal blood flow; and RVR, renal vascular resistance. Values are mean±SEM.

*P<0.05 vs control (n=10).

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**Figure 1.** The effect of NLA (30 mg/kg) on the change in renal blood flow (top) and in renal vascular resistance (bottom) expressed as a percentage of baseline values. At baseline, renal blood flow and renal vascular resistance were 259±20 mL/min and 0.229±0.016 mm Hg · mL⁻¹ · min⁻¹, respectively (n=10).

*P<0.05 vs baseline.
Effects of NOS Inhibition on Blood Gases and Hematocrit

The pH, P CO ₂, P O ₂, oxygen content, and hematocrit of the arterial and venous blood samples before and at 20 and 60 minutes after NLA are shown in Table 3. Throughout the experimental period, arterial and renal venous blood gases remained within the physiological range; however, administration of NLA caused 0.48 ± 0.07% and 0.60 ± 0.14% reductions in arterial and renal venous pH, respectively, at 60 minutes. This was concomitant with 14 ± 3% and 11 ± 4% decreases in arterial and renal venous P CO ₂. Renal venous P O ₂ values decreased significantly at all time points examined after NLA administration: at 60 minutes, renal venous P O ₂ was 14 ± 3% lower than control. The arterial oxygen content appeared to increase after NLA administration ( P < 0.05); however, this occurred in parallel with a significant increase in hematocrit.

Effects of NOS Inhibition on Oxygen Uptake, Extraction, and Consumption

Oxygen uptake and extraction were significantly increased above baseline by 20 minutes after NLA administration and increased by 115 ± 37% and 102 ± 34%, respectively, from baseline after 60 minutes (Figure 2). Oxygen consumption was calculated in order to account for the alterations in renal blood flow induced by NLA and is displayed in absolute units and as percentage change from control in Figure 3. Most important, NLA administration significantly increased renal oxygen consumption at all time points examined.

### Table 2. Effects of NOS Inhibition, With NLA, on Renal Function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Time After NLA, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>109 ± 12</td>
<td>95 ± 11</td>
</tr>
<tr>
<td>UV, mL/min</td>
<td>0.48 ± 0.07</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>FR Na, %</td>
<td>99.6 ± 0.1</td>
<td>99.7 ± 0.1</td>
</tr>
<tr>
<td>R Na, mmol/min</td>
<td>16 ± 1.7</td>
<td>13 ± 1.6</td>
</tr>
</tbody>
</table>

FR Na indicates fractional reabsorption of sodium; R Na, amount of sodium reabsorbed. Values are mean ± SEM.

* P < 0.05 vs control (n = 9 or 10).

### Table 3. Effects of NOS Inhibition, With NLA, on Arterial and Renal Venous Blood Gases

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Time After NLA, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7.454 ± 0.012</td>
<td>7.469 ± 0.016</td>
</tr>
<tr>
<td>V</td>
<td>7.447 ± 0.014</td>
<td>7.456 ± 0.016</td>
</tr>
<tr>
<td>P CO ₂, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>37.2 ± 0.9</td>
<td>34.6 ± 1.0*</td>
</tr>
<tr>
<td>V</td>
<td>38.3 ± 1.2</td>
<td>36.0 ± 1.3</td>
</tr>
<tr>
<td>P O ₂, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>86.3 ± 1.6</td>
<td>85.4 ± 2.8</td>
</tr>
<tr>
<td>V</td>
<td>54.4 ± 1.1</td>
<td>50.5 ± 1.1*</td>
</tr>
<tr>
<td>O₂CT, Vol%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>15.2 ± 0.6</td>
<td>15.6 ± 0.6</td>
</tr>
<tr>
<td>V</td>
<td>13.5 ± 0.5</td>
<td>12.9 ± 0.6</td>
</tr>
<tr>
<td>HCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>37.7 ± 1.2</td>
<td>38.4 ± 1.2</td>
</tr>
</tbody>
</table>

O₂CT indicates oxygen content; HCT, hematocrit; A, arterial; and V, venous. Values are mean ± SEM.

* P < 0.05 vs control (n = 10).
Effects of NOS Inhibition on the Relationship Between Oxygen Consumption and Sodium Reabsorption by the Kidney

The main ATP-consuming process of the kidney is sodium reabsorption; hence, in the normal kidney, oxygen consumption is linearly related to sodium reabsorption. When the oxygen consumption was plotted against the amount of sodium reabsorbed for each of the time points examined, NLA shifted the point both leftward and upward (Figure 4). For instance, at 60 minutes after NLA, the $O_2$ consumed per millimole of sodium reabsorbed had increased by $160\pm34\%$ ($P<0.05$).

Effects of NOS Inhibition on Renal Handling of NOx

NLA had no significant effect on arterial plasma concentrations of NOx (Figure 5, top); however, it appeared to decrease the filtered load of NOx from $0.527\pm0.115$ to $0.475\pm0.088$ and $0.429\pm0.117$ $\mu$mol/min at 20 and 60 minutes after NLA administration, respectively ($P=NS$). Moreover, the fractional reabsorption of NOx increased from $89\pm3.5\%$ to $93\pm1.6\%$ at 20 minutes and to $94\pm1.4\%$ at 60 minutes after NLA administration (both $P<0.05$ versus control), maintaining the amount of NOx reabsorbed by the kidney and thereby tending to reduce the urinary concentration of NOx (Figure 5, middle panel). This, in combination with the reduction in UV caused by NLA administration, resulted in a significant reduction in excreted NOx (Figure 5, bottom).

Effects of NO on Renal Oxygen Consumption In Vitro

Figure 6 illustrates the effects of increasing doses of SNAP and bradykinin on the oxygen consumption of canine cortical and medullary slices. The levels of baseline oxygen consumption for cortical and medullary slices were not different from one another ($344\pm36$ versus $324\pm46$ $nmol$ $O_2$ $\cdot$ min$^{-1}$ $\cdot$ g$^{-1}$, respectively; $P=NS$). SNAP dose-dependently decreased the oxygen consumption of both cortical and medullary slices. At the highest dose of SNAP, oxygen consumption was decreased from baseline by $44\pm4\%$ and $40\pm6\%$ for cortical and medullary slices, respectively. Moreover, this effect of SNAP was not altered by L-NAME. Bradykinin also reduced oxygen consumption of both cortical and medullary renal slices: at $10^{-4}$ mol/L, bradykinin reduced cortical and medullary oxygen consumption by $28\pm4\%$ and $24\pm2\%$ from control ($P<0.05$). This effect was attenuated by L-NAME ($10^{-4}$ mol/L).

Discussion

The present study has demonstrated that inhibition of NOS produced a doubling of oxygen consumption in spite of a reduction in sodium reabsorption, the main ATP-consuming process in the kidney. This marked increase in the amount of oxygen consumed per unit of sodium reabsorbed after NOS inhibition suggests an important and tonic role for NO in maintaining the balance between oxygen consumption and the chemical work performed by the kidney in conscious dogs. In contrast, release of NO either chemically, using SNAP, or from renal tissue, using bradykinin, resulted in a reduction of either renal cortical or medullary oxygen consumption in vitro. These data directly support a role for nitric oxide in the control of renal oxygen consumption.
The dose of NLA used in this study (30 mg/kg) has been used previously by us to inhibit NOS,\textsuperscript{9,11} and the hemodynamic response was consistent with that reported by others\textsuperscript{15,16}; i.e., blockade of NOS resulted in a significant increase in arterial blood pressure and a decrease in heart rate. A significant reduction in arterial PO\textsubscript{2}, which also occurs as a result of NOS inhibition, was also noted.\textsuperscript{15,17} Moreover, the observed decrease in renal blood flow and increase in renal vascular resistance, which have previously been described,\textsuperscript{15,18} suggest that a significant degree of renal NOS inhibition was attained.

NOS inhibition produced a marked reduction in UV and sodium excretion, a finding consistent with previous studies in anesthetized dogs and rats.\textsuperscript{18,19} This reduction in sodium excretion was associated with a reduction in the filtered load of sodium brought about by the significant reduction in GFR. Such a reduction in GFR in response to NOS inhibition was also found after the administration of L-NAME to conscious dogs\textsuperscript{20} and with intrarenal infusions of N\textsuperscript{G}-monomethyl-L-arginine in anesthetized dogs.\textsuperscript{21} A reduction in GFR has also been noted in conscious rabbits\textsuperscript{22} and in isolated perfused rat kidneys\textsuperscript{23} after NLA administration. However, this is not a consistent finding throughout the literature, and a number of studies have reported little or no change in GFR after NOS inhibition.\textsuperscript{15,18} In that context, a significant reduction in GFR in the present study was not noted until 60 minutes after the administration of NLA, indicating that this effect may involve a time-dependent mechanism. On the other hand, the increase in oxygen consumption paralleled the increase in vascular resistance and arterial pressure, both attributable to the inhibition of NO synthesis. Thus, despite the late change in GFR (at 60 minutes), there was a marked shift in the ratio of sodium reabsorbed to oxygen consumption within 20 minutes, suggesting a GFR-independent alteration in renal oxygen consumption.

The amount of sodium reabsorbed decreased by almost 50% after NOS inhibition in the present study. NO has been reported to have an inhibitory effect on proximal tubule sodium transport;\textsuperscript{24} hence, NOS inhibition would have been expected to increase sodium reabsorption. The present study was carried out on animals 18 hours after their last dietary intake of sodium; thus, dogs were in a state of sodium conservation, as illustrated by their almost maximal fractional reabsorbance. Since NOS inhibition in these animals failed to affect the fractional reabsorption of sodium, the reduction in the molar amounts of sodium reabsorbed can be directly ascribed to the reduction in GFR leading to a reduction in the filtered load of sodium. Whatever the mechanism for the reduction in the molar amount of sodium reabsorbed, a consequent reduction in oxygen consumption would have been predicted.

Oxygen consumption by the kidney, in marked contrast to sodium reabsorption, increased almost 2-fold after NOS inhibition. This resulted in a marked increase in the amount of oxygen consumed per mole of sodium reabsorbed (Figure 4) and would markedly alter the linear
relationship between oxygen consumption and sodium reabsorption. Whether this increase in oxygen consumption after NOS inhibition was due to an increase in the basal oxygen consumption, which would result in an upward shift in the relationship with the same slope, or was the result of a change in the efficiency of the reabsorption of sodium, which would simply increase the slope of the relationship, was beyond the scope of the present study. Our study offers no information on whether the increase in oxygen consumption after NLA is directly or indirectly coupled to sodium reabsorption. Nevertheless, we have demonstrated that NO serves to maintain the relationship between oxygen consumption and the ATP-dependent process of sodium reabsorption in the kidneys of conscious dogs.

Although the main ATP-consuming process of the kidney is sodium reabsorption, it is conceivable that administration of NLA caused an increase in yet another ATP-dependent process, which would account for the increase in oxygen consumption. Indeed, a minor respiratory alkalosis of 0.036 pH units that would be expected to be compensated for by an increase in bicarbonate excretion occurred by 60 minutes after NLA administration. Although this may be due to the increase in body temperature after NLA, as we have previously described, neither body temperature nor respiratory frequency (hyperventilation) was measured in the present study. However, it is the process of bicarbonate reabsorption that is partially dependent on the operation of the ATP-consuming proton pump and so any decrease in bicarbonate reabsorption, leading to excretion, would be expected to reduce the ATP utilization and, thus, oxygen consumption. This would not explain the increase in oxygen consumption observed. Moreover, NLA decreased GFR, thus decreasing the filtered load of bicarbonate from 2.7 to 1.5 mmol/min at 60 minutes. Since, normally, almost 100% of bicarbonate is reabsorbed, it is difficult to perceive that treatment with NLA would result in an increased amount of bicarbonate reabsorbed from a smaller volume of glomerular filtrate. Indeed, a reduction in the ATP cost of this process would be predicted at a point where we measured an increase in renal oxygen consumption.

It is also possible that NLA induced an alteration in the substrate utilization of the kidney, i.e., a switch to a more inefficient fuel. For example, switching from the most efficient substrate, glucose (which theoretically produces 3 moles of ATP per mole of atomic oxygen), to the least efficient, fatty acids (palmitate produces only 2.6 moles of ATP per mole of atomic oxygen), would result in a 15% increase in the oxygen consumed for any given level of ATP used. Such a switch, however, does not explain the 90% increase in oxygen consumption observed in the present study. Moreover, when the respiratory quotient was calculated for the kidney in the present study, it was found to increase from 0.7 to 1.1 at the point of maximum oxygen consumption, indicating a switch toward carbohydrate metabolism. This would cause an underestimation of the magnitude of the increase in oxygen consumed in relation to the chemical work performed.

It could be argued that NLA had additional effects as well as NOS inhibition in the present study. Indeed, NLA specifically has been reported to have an inhibitory action on ATP-sensitive potassium channels in cat and rat pial arterioles. If NLA had this effect on renal ATP-sensitive potassium channels in the present study, a reduction in the reabsorption of sodium by the proximal tubules and a concomitant diuresis would be predicted. In contrast, a reduction in UV was observed after NLA administration; moreover, the fractional reabsorption of sodium remained unchanged, suggesting no functional consequence of this proposed effect of NLA in the present study. Nevertheless, if NLA did reduce sodium reabsorption, then a reduction in the ATP utilization of the kidney would also occur and would still not explain the almost 2-fold increase in renal oxygen consumption that we observed.

We have also demonstrated that NO administered either exogenously, using a NO donor, SNAP, or generated endogenously, via administration of the NOS-stimulating agonist bradykinin, produces dose-dependent decreases in the oxygen consumption of canine renal cortical and medullary slices in vitro. This lends further support to the in vivo data indicating that NO inhibits renal oxygen consumption, as evidenced by an increase in renal oxygen consumption for any given level of chemical work after blockade of NOS. It should be noted that NLA by itself had no effect on renal tissue oxygen consumption in vitro (Figure 6). We have observed this previously in other tissues, including the heart, and believe that there are no agonists or flow to stimulate NO production in our tissues in vitro. Thus, inhibition of NO synthesis does not alter baseline tissue oxygen consumption in vitro. This is in contrast to our in vivo studies, in which both flow shear stress and agonists that stimulate NO production are always present. It is not the antagonist, L-NAME or NLA, or the route of administration, local or systemic, that determines the results but rather the presence of agonists or blood flow.

Such a role for NO has also been demonstrated in vivo in the whole body, in skeletal muscle, and across the heart of conscious dogs. Moreover, exogenous application of NO donors or release of NO by agonists in cardiac and skeletal muscle in vitro also reduces oxygen consumption. The mechanism via which NO reduces oxygen consumption was not assessed in the present study; however, a number of studies have demonstrated a direct reversible interaction of NO with cytochrome oxidase of the electron transport chain in the mitochondria. Moreover, peroxynitrite, the product of the reaction of NO with the superoxide radical, has been shown to inhibit complex I and II of the electron transport chain; however, this inhibition is generally thought to be irreversible.

In previous studies in skeletal and heart muscle in vivo, there was an increase in oxygen consumption at any level of mechanical work during exercise after inhibition of NO synthesis. These studies suggested that inhibition of NO synthesis may alter mechanical efficiency in muscle. However, efficiency is often defined as energy produced per unit oxygen consumed. To directly address this was beyond the scope of our previous studies and is technically difficult. The ATP-dependent sodium/potas-
sium pump is the main energy-consuming process in the kidney, and Deetjen\(^\text{12}\) has shown that sodium transport is linearly related to oxygen consumption over a wide range of metabolic work. Hence, we used the amount of sodium reabsorbed in the kidney as an index of this chemical work, and we found a marked shift upward and to the left in the relationship between sodium reabsorbed and oxygen consumed. This indicates a marked reduction in the chemical efficiency of the kidney after inhibition of NO synthesis. Our study is then, to our knowledge, the first to demonstrate that NO plays a role in maintaining a balance between oxygen consumption and chemical work in the kidney and extends our studies on the role of NO in the regulation of mechanical work in heart and skeletal muscle.

Administration of NOS inhibitors has been reported to cause an increase in sympathetic nerve activity to the kidney\(^\text{29}\); however, if this did occur in the present study, the increase in sympathetic activity would be expected to increase sodium reabsorption\(^\text{30}\) and would result in an underestimation of the reduction of sodium reabsorption caused by NOS inhibition. Moreover, norepinephrine has been demonstrated to increase Na\(^+\),K\(^+\)-ATPase activity in microsomes isolated from rat proximal tubules without a concomitant increase in oxygen consumption,\(^\text{31}\) an effect that would again result in an underestimation of the increase in the amount of oxygen consumed per mole of sodium reabsorbed in the present study.

Another important finding of the present study was that NOS inhibition resulted in a marked reduction in the excretion rate of NO\(_x\), the sum of the amount of dissolved NO and its metabolic products, nitrite and nitrate. This finding is consistent with the study by Majid et al.,\(^\text{32}\) in which intrarenal NOS inhibition resulted in a marked reduction in the excretion of NO\(_x\) in urine.\(^\text{29}\) Anesthetized dogs. In this and other studies,\(^\text{33}\) the concentration of NO\(_x\) in urine has been used as an indicator of NO production acutely. Moreover, a reduction in NO\(_x\) excretion as a result of intrarenal NOS inhibition has been used as evidence for tonic production of NO by the kidney. However, the biological half-life of NO\(_x\) has been demonstrated to be 3.8 hours in conscious dogs\(^\text{31}\); ie, if NO production was instantly blocked completely, one would not observe a 50% reduction in excretion for 3.8 hours. Since 3.8 hours is much longer than our experimental time, it suggests that the reduction in the excretion of NO\(_x\) in the present study cannot, in the main, be due to a reduction in the production of NO. Indeed, despite systemic NOS inhibition, arterial plasma concentrations of NO\(_x\) were not altered significantly over the course of our experiment. Therefore, the reduction in the urinary excretion rate of NO\(_x\) by NOS inhibition was, in fact, secondary to a reduction in the filtered load of NO\(_x\), a result of the reduction in GFR, in combination with a significant increase in the fractional reabsorption of NO\(_x\). This finding highlights the importance of considering the biological half-life of NO\(_x\) when using nitrate excretion as an indicator of renal NO production.

In conclusion, since NOS inhibition markedly increases renal oxygen consumption in spite of a decrease in the chemical work performed and NO reduces both renal cortical and medullary oxygen consumption in vitro, we have demonstrated that NO plays a key role in regulating the balance between sodium reabsorption, the main ATP-consuming process, and oxygen consumption in the kidney of conscious dogs.

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References


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