Renal hemodynamic and excretory responses to intra-arterial infusion of peroxynitrite in anesthetized rats

Luis C. Matavelli,1 Philip J. Kadowitz,2 L. Gabriel Navar,1 and Dewan S. A. Majid1

Departments of 1Physiology and 2Pharmacology, Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, Louisiana

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Matavelli LC, Kadowitz PJ, Navar LG, Majid DS. Renal hemodynamic and excretory responses to intra-arterial infusion of peroxynitrite in anesthetized rats. Am J Physiol Renal Physiol 296: F170–F176, 2009. First published November 5, 2008; doi:10.1152/ajprenal.90487.2008.—Peroxynitrite (ONOO−) is formed endogenously by the reaction of nitric oxide (NO) and superoxide (O2−). To examine the hypothesis that ONOO− causes renal vasodilation at low concentrations but causes vasoconstriction at higher concentrations, we examined renal responses to intra-arterial infusion of incremental doses of ONOO− (10, 20, and 40 μg·kg−1·min−1; 45 min each) in anesthetized rats. Renal blood flow (RBF) and glomerular filtration rate (GFR) were determined by PAH and inulin clearance. In control rats (n = 6), low dose (10 μg·kg−1·min−1) of ONOO− increased RBF by 10 ± 3% and GFR by 15 ± 5%. The higher doses (20 and 40 μg·kg−1·min−1) mostly reversed these responses which were −7 ± 4% and −27 ± 7% (P < 0.05) in RBF and −0.1 ± 4.8% and −14 ± 12% in GFR, respectively. There were no appreciable changes in urine flow (V) and sodium excretion (UnaV) during ONOO− infusion. However, in rats pretreated with NO synthase (NOS) inhibitor, L-NAME (50 μg·kg−1·min−1; n = 5), these doses of ONOO− significantly reduced RBF (−26 ± 7, −27 ± 6, and −44 ± 3%) and GFR (−21 ± 6, −25 ± 8, and −32 ± 12%) with variable increases in V or UnaV. Long-term infusion of ONOO− (10 μg·kg−1·min−1 for 75 min) in another set of control rats (n = 5) also showed similar vasodilator and hyperfiltration responses. These data indicate that ONOO− acts as an oxidant at high concentration but provides renoprotective function at low concentration that depends on intact NOS activity.

NITRIC OXIDE (NO) reacts with superoxide (O2−) nonenzymatically to generate peroxynitrite (ONOO−), a powerful oxidant molecule. Although the discovery of ONOO− was reported many years ago (23), its biologic oxidant activity has been recognized only in recent years (3). ONOO− can induce cytotoxic effects by several mechanisms, including sulfhydryl oxidation, protein tyrosine nitration, membrane lipid peroxidation, as well as DNA damage leading to cellular injury and death (6, 32, 33). Indeed, the overproduction of ONOO− may occur in a variety of pathologic conditions causing major cytotoxic effects either by direct or indirect oxidant mechanisms (1, 4, 36). In contrast, it has also been proposed that, under physiological conditions, the production of ONOO− will be low and oxidative damage minimized by endogenous antioxidant defense mechanisms (34).

Prior animal experiments addressing mechanisms of oxidative stress associated with renal dysfunction and hypertension have strongly suggested a renoprotective role for the NO−O2− interaction (13, 17, 20–22, 27, 28), but the exact mechanism associated with this protection has not yet been determined. It is hypothesized that ONOO− is a major factor involved in this protection, although its cytotoxic effects would argue against a renoprotective role. However, cytotoxic effects usually occur at high concentrations (micromolar to millimolar) as shown in vitro studies (31). At low concentrations (nanomolar to low micromolar), ONOO− induces vascular relaxation, which may be physiologically more relevant, as previously demonstrated by in vivo studies (19, 30). It is possible that the vasodilatory effects of ONOO− at low concentrations are mediated either by a reverse nonenzymatic reaction of ONOO− to NO and O2−, or by the conversion of ONOO− to a NO donor compound (31). It is postulated that nitrite, a major end product of ONOO−, acts as a NO donor by its reduction to NO (14). This reaction occurs in certain specific conditions and is mediated by a nitrite reductase pathway involving the endothelium (35).

Although the biological activity of ONOO− has been extensively investigated in recent years, few in vivo studies have been conducted to examine its direct effects on hemodynamics and organ function. In particular, the role of direct ONOO− administration on renal excretory function has not been reported. Thus, the present study was designed to evaluate renal hemodynamic and excretory responses to acute ONOO− infusion at different doses directly into the renal artery, in the presence or absence of the NO synthase (NOS) enzyme inhibitor nitro-l-arginine methyl ester (l-NAME) in rats.

METHODS

Experiments were performed on male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 300 to 350 g. The protocol was approved by the Tulane University Animal Care and Use Committee. The rats were given food and tap water ad libitum and a minimum of 1 wk was allowed for them to adjust to the animal care facility before conducting acute renal clearance experiments to determine renal responses to infusions of incremental doses of ONOO−. Solutions of ONOO− in different concentrations were prepared from a stock solution of sodium peroxynitrite (purchased from Cayman Chemicals, Ann Arbor, MI) which was supplied as a solution in sodium hydroxide. This solution remained stable for 7% (P < 0.05) in RBF and −0.1 ± 4.8% and −14 ± 12% in GFR, respectively. There were no appreciable changes in urine flow (V) and sodium excretion (UnaV) during ONOO− infusion. However, in rats pretreated with NO synthase (NOS) inhibitor, L-NAME (50 μg·kg−1·min−1; n = 5), these doses of ONOO− significantly reduced RBF (−26 ± 7, −27 ± 6, and −44 ± 3%) and GFR (−21 ± 6, −25 ± 8, and −32 ± 12%) with variable increases in V or UnaV. Long-term infusion of ONOO− (10 μg·kg−1·min−1 for 75 min) in another set of control rats (n = 5) also showed similar vasodilator and hyperfiltration responses. These data indicate that ONOO− acts as an oxidant at high concentration but provides renoprotective function at low concentration that depends on intact NOS activity.

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omometer (Bio-Rad Laboratories, Hercules, CA). Immediately before use, stock solution concentration was calculated using the extinction coefficient for ONOO\textsuperscript{−} of 1.670 M/cm. Before intra-arterial infusion, an aliquot of the ONOO\textsuperscript{−} stock solution (generally in the range of 25 to 35 nM) was diluted with cold NaOH solution to maintain an alkaline pH and infused with a cold glass syringe. The pH of the final infusion solution was 8.0. To prevent degradation of ONOO\textsuperscript{−} in solution before infusion into the renal artery, syringe and infusion catheters were kept in ice-jacket during the period of infusion. At the end of each experiment, the activity of residual ONOO\textsuperscript{−} was tested spectrophotometrically.

Experiments

Protocol 1. Rats were randomly divided into two groups, based on pretreatment with or without NOS inhibitor L-NAME (Sigma, St. Louis, MO). Rats in group 1 were not given L-NAME (control rats; n = 6) and rats in group 2 were pretreated with L-NAME (L-NAME-treated rats; n = 5) before infusions of ONOO\textsuperscript{−} doses. On the day of experiment, rats were anesthetized with thiobutabarbital sodium (100 mg/kg ip Inactin; Sigma) and placed on a heating pad to maintain rectal temperature at 37°C throughout the study. After tracheal intubation, the right jugular vein was catheterized for intravenous administration of saline solutions containing albumin (bovine serum, Calbiochem, La Jolla, CA), para-aminomhippuric acid (PAH; MP Biomedicals, Aurora, OH), and inulin (Inutest; Laevosan, Linz/Donau, Austria). The right femoral artery was cannulated for continuous monitoring of arterial pressure, using a Biopac MP100 data-acquisition system, and for arterial blood samplings. Thereafter, the left kidney was exposed via a flank incision and placed in a Lucite cup, the right renal artery was isolated, and the left ureter was cannulated for urine collection. A tapered PE-10 catheter was then inserted into the renal artery via the left femoral artery to allow intra-arterial administration of drugs (ONOO\textsuperscript{−} doses) directly into the kidney (20, 22). This catheter was kept patent by a continuous infusion of heparinized isonic saline at a rate of 5 μL/min.

After a 60-min period for stabilization following completion of surgical procedures, the experimental protocol was started with a 30-min control clearance period to assess baseline renal hemodynamic and excretory parameters. This initial period was considered the baseline period in group 1. In group 2, following 30-min initial control clearance period, L-NAME was infused intra-arterially for 60 min and then stopped. The baseline period for ONOO\textsuperscript{−} infusion in this group was considered the last 30-min period of L-NAME infusion. In both groups, ONOO\textsuperscript{−} was then infused in three incremental doses of 10, 20, and 40 μg·kg\textsuperscript{−1}·min\textsuperscript{−1} administered intra-arterially. At the beginning of each rate of infusion, a minimum of 15 min was allowed for stabilization followed by a 30-min clearance period for sample collections. In each experiment, a new ONOO\textsuperscript{−} solution was prepared immediately before each period of infusion.

These doses of ONOO\textsuperscript{−} were selected based on results from pilot studies using a wide range of doses. These selected doses were within the range that showed a stable systemic blood pressure and physical state of the animals during these intrarenal administrations. It is of note that the highest dose of ONOO\textsuperscript{−} (100 μg·kg\textsuperscript{−1}·min\textsuperscript{−1}) tested in these pilot experiments caused severe hematologic disturbances such as hemolysis, renal artery thrombosis, and hematuria. Additionally, to verify the activity of the ONOO\textsuperscript{−} solution, further experiments were carried out with ONOO\textsuperscript{−} solutions that had been allowed to degrade for 1 to 3 wk at room temperature. The doses administered for degraded ONOO\textsuperscript{−} were exactly the same as the doses of active stable ONOO\textsuperscript{−} at 10, 20, and 40 μg·kg\textsuperscript{−1}·min\textsuperscript{−1}. These degraded solutions were also prepared from the stock solution of degraded compound, which was the same concentration as of the stable compound. The solutions of degraded ONOO\textsuperscript{−} also had a similar pH (8.0) as stable compounds. These experiments were used as the time control experiments with proper vehicle treatment. In these experiments, the degraded ONOO\textsuperscript{−} had no significant renal or systemic effects. Since infusion of the degraded compound did not cause any significant change in renal parameters, the data indicate that the use of small volume of alkaline solutions (infusion rate 0.5 μL/min) was effectively buffered in the plasma during intra-arterial administration. Furthermore, a few time-controlled experiments were performed in L-NAME-pretreated animals (n = 3) without administration of the ONOO\textsuperscript{−} solution. In these experiments, L-NAME was infused for 60 min as previously described and then saline, instead of ONOO\textsuperscript{−}, was infused for more than a 90-min period. These time control experiments were conducted to evaluate the responses to ONOO\textsuperscript{−} infusion observed in L-NAME-treated rats.

Protocol 2. To explore a possible or delayed effect on the kidney, a low dose of ONOO\textsuperscript{−} (10 μg·kg\textsuperscript{−1}·min\textsuperscript{−1}) was administered in control rats (n = 5) for a period of 75 min longer than that used in protocol 1. These control rats were subjected to the same surgical procedures as described earlier. Additionally, an ultrasonic flow probe (Transonic System) was placed around the left renal artery to continuously monitor renal blood flow (RBF) and further confirm the RBF responses to ONOO\textsuperscript{−} infusion observed using the PAH clearance technique. In these experiments, the protocol was started with two 30-min control clearance periods to assess baseline hemodynamic and excretory parameters. ONOO\textsuperscript{−} was then infused intra-arterially for a 75-min period. After the initiation of ONOO\textsuperscript{−} infusion, a 15-min period was allowed for stabilization followed by two 30-min periods for clearance collections. As in the other experiment protocol, a new ONOO\textsuperscript{−} solution was prepared immediately before each period of infusion.

Analytical Procedures

The collected blood and urine samples were analyzed for inulin, PAH, and sodium/potassium concentrations. Inulin and PAH concentrations were determined by spectrophotometry and sodium/potassium concentrations were determined by flame photometry. The value for inulin clearance was considered as glomerular filtration rate (GFR) and the value for PAH clearance was considered as renal plasma flow (RPF). The formulas for measurements of GFR and RPF are as follows: GFR (inulin clearance) = (urinary conc. of inulin × urine volume)/plasma inulin conc.; RPF (PAH clearance) = [(urinary conc. of PAH × urine volume)/plasma PAH conc.]. RBF was calculated from RPF and hematocrit (Hct) value [RBF = RPF/(1 – Hct)]. Renal vascular resistance (RVR) was calculated by dividing the value of mean arterial pressure (MAP) with the value of RBF. Urinary concentration of 8-isoprostane was measured by enzyme immunoassay (Cayman Chemical) and nitrate/nitrite were determined using a colorimetric assay kit (Cayman Chemical). All values were normalized per gram of kidney weight.

Statistical Analysis

Data are expressed as means ± SE. In experiments in protocol 1, comparisons between baseline values and the values obtained during infusion of ONOO\textsuperscript{−} doses in both groups were made using one-way repeated-measures ANOVA followed by a Bonferroni test for post hoc comparisons. Comparison of the percent responses to ONOO\textsuperscript{−} infusion among the experimental groups (control and L-NAME-treated) was made using unpaired Student’s t-test. In experiments in protocol 2, responses to single-dose ONOO\textsuperscript{−} infusion were compared with baseline values using paired Student’s t-test. P < 0.05 was considered to be statistically significant.

RESULTS

Protocol 1 Experiments

Tables 1 and 2 show the absolute responses to ONOO\textsuperscript{−} infusion in control (group 1) and in L-NAME-treated (group 2)
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Table 1. Responses to incremental doses of ONOO− in intact rats (n = 6) untreated with L-NAME (group 1, protocol 1)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>10</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>128±3</td>
<td>131±2</td>
<td>130±3</td>
<td>132±3</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·g⁻¹</td>
<td>6.6±0.6</td>
<td>7.3±0.7</td>
<td>6.1±0.5</td>
<td>4.4±0.8*</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min·g⁻¹</td>
<td>20.3±2.1</td>
<td>18.9±2.2</td>
<td>22.1±2.0</td>
<td>35.1±6.3*</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g⁻¹</td>
<td>1.02±0.12</td>
<td>1.17±0.14</td>
<td>1.00±0.12</td>
<td>0.84±0.14</td>
</tr>
<tr>
<td>V, µl·min⁻¹·g⁻¹</td>
<td>10.2±3.5</td>
<td>10.9±3.3</td>
<td>8.6±2.0</td>
<td>10.7±3.4</td>
</tr>
<tr>
<td>UNOxV, µmol·min⁻¹·g⁻¹</td>
<td>0.9±0.3</td>
<td>0.5±0.3</td>
<td>0.7±0.2</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>UNaV, mmol·min⁻¹·g⁻¹</td>
<td>1.2±0.6</td>
<td>1.8±0.7</td>
<td>2.0±0.7</td>
<td>2.7±1.3</td>
</tr>
<tr>
<td>IISOV, pg·min⁻¹·g⁻¹</td>
<td>5.0±1.5</td>
<td>4.1±1.4</td>
<td>5.5±1.6</td>
<td>5.9±1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; statistical analyses were conducted using one-way repeated-measures ANOVA followed by a Bonferroni test for post hoc comparisons. ONOO−, peroxynitrite; L-NAME, N⁵-nitro-L-arginine methyl ester; MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; U, urine flow; UNaV, urinary sodium excretion rate; UNOxV, urinary nitrate/nitrite excretion rate; IISOV, urinary isoprostane excretion rate. *P < 0.05 vs. baseline.

Table 2. Responses to incremental doses of ONOO− in L-NAME-treated rats (n = 5; group 2, protocol 2)

<table>
<thead>
<tr>
<th></th>
<th>Baseline, 50 µg·kg⁻¹·min⁻¹ L-NAME</th>
<th>10</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>123±2</td>
<td>131±2</td>
<td>132±3</td>
<td>130±3</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·g⁻¹</td>
<td>7.3±0.7</td>
<td>4.2±0.4†</td>
<td>4.2±0.4†</td>
<td>3.4±0.5†</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min·g⁻¹</td>
<td>17.4±1.7</td>
<td>32.4±3.8†</td>
<td>32.4±4.1</td>
<td>40.2±5.9†</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g⁻¹</td>
<td>1.35±0.10</td>
<td>1.00±0.11†</td>
<td>0.94±0.12†</td>
<td>0.85±0.17†</td>
</tr>
<tr>
<td>V, µl·min⁻¹·g⁻¹</td>
<td>7.4±1.0</td>
<td>14.6±6.2</td>
<td>12.8±4.0</td>
<td>12.4±3.5</td>
</tr>
<tr>
<td>UNOxV, µmol·min⁻¹·g⁻¹</td>
<td>0.7±0.2</td>
<td>1.3±0.4</td>
<td>1.5±0.3</td>
<td>1.6±0.3†</td>
</tr>
<tr>
<td>UNaV, mmol·min⁻¹·g⁻¹</td>
<td>1.7±0.2</td>
<td>1.7±0.6</td>
<td>2.5±1.0</td>
<td>2.6±1.0</td>
</tr>
<tr>
<td>IISOV, pg·min⁻¹·g⁻¹</td>
<td>3.9±1.6</td>
<td>4.9±1.7</td>
<td>5.0±1.5</td>
<td>4.4±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; statistical analyses were conducted using one-way repeated-measures ANOVA followed by a Bonferroni test for post hoc comparisons. *P < 0.05 vs. control; †P < 0.05 vs. baseline values before ONOO− infusion (L-NAME period).

As the baseline values before ONOO− infusions are different between group 1 and group 2, the responses to ONOO− are normalized and expressed as percent change from the baseline value which is taken as 100%. Figures 1, 2, 3, and 4 illustrate the percent responses to ONOO− in both groups of animals. L-NAME administration in group 2 decreased RBF and increased MAP and RVR without significant changes in GFR, urine flow (V), sodium excretion (UNaV), or fractional excretion of sodium (FENa, Table 2). L-NAME infusion decreased urinary excretion rate of nitrite/nitrate (UNOxV) and increased 8-isoprostane excretion rate (IISOV) as reported earlier (21, 22, 27, 28).

ONOO− infusion directly in the renal artery did not cause a change in MAP in both groups of rats. However, low-dose infusion of ONOO− (10 µg·kg⁻¹·min⁻¹) in control rats caused significant increases of 10 ± 3% in RBF and of 15 ± 5% in GFR with a decrease of 7 ± 2% in RVR as illustrated in Figs. 1–3. There were no significant changes in RBF (−7 ± 4%), GFR (−0.1 ± 4.8%), or RVR (10 ± 5%) at the mid-dose infusion of ONOO− (20 µg·kg⁻¹·min⁻¹). With high-dose (40 µg·kg⁻¹·min⁻¹) infusion, ONOO− caused a significant reduction in RBF (−27 ± 7%) and an increase in RVR (50 ± 18%) without a significant change in GFR (−14 ± 12%; Figs. 1–3). There were no significant changes in V (18 ± 12, 8 ± 17, and 13 ± 10%), UNaV (−1 ± 10, −10 ± 9, and 39 ± 19%), or in FENa (−11 ± 12, −9 ± 14, and 79 ± 41%) during infusion of increasing doses of ONOO−. In the experiments in group 1, UNOxV and IISOV were measured in three rats as urine samples in the initial three rats were not collected for these purposes. UNaV showed increasing trend with increasing doses of ONOO− (67 ± 19, 100 ± 62, and 189 ± 136%). In contrast, IISOV was decreased (−20 ± 10%) with the lowest dose of ONOO− but had an increasing trend (13 ± 12 and 22 ± 10%) with the higher doses.

In L-NAME-treated rats (group 2), ONOO− infusion elicited significant decreases in RBF (−26 ± 7, −27 ± 6, and −44 ± 3%) and GFR (−21 ± 6, −25 ± 8, and −32 ± 12%) and increases in RVR (41 ± 14, 42 ± 13, and 79 ± 12%) with increasing doses of ONOO− infused (Figs. 1–3). However, ONOO− infusion in L-NAME-treated rats produced variable increases in V (60 ± 24, 58 ± 27, and 57 ± 27%), UNaV (59 ± 25, 125 ± 67, and 152 ± 88%), and FENa (110 ± 47, 230 ± 113, and 409 ± 283%). Figure 4 illustrates the percent changes in UNaV in these rats. Incremental doses of ONOO− infusion also produced variable increases in IISOV (39 ± 21, 92 ± 64, and 108 ± 68%). Although IISOV increased significantly due to L-NAME treatment, doses of ONOO− infusion did not cause further significant changes in IISOV (Table 2). Compared with these results with infusion of ONOO− doses, infusion of the vehicle in L-NAME-treated rats was without such marked changes in these hemodynamic and excretory parameters. During these three collection periods during vehicle infusion in L-NAME-treated rats (n = 3), the changes in mean absolute values are as follows: RBF 5.6 ± 0.7 to 5.6 ± 0.9, 4.8 ± 0.5, 4.9 ± 0.5 ml·min⁻¹·g⁻¹; GFR 1.0 ± 0.2 to 0.94 ± 0.2, 0.94 ± 1.1 ± 0.1 ml·min⁻¹·g⁻¹; RVR 28.8 ± 9.1 to 33.3 ± 12.2, 32.3 ± 7.9, 30.7 ± 6.3 mmHg·ml⁻¹·min⁻¹·g⁻¹; V 5.7 ± 1.5 to 7.3 ± 3.0, 6.6 ± 2.1, 5.2 ± 0.8 µl·min⁻¹·g⁻¹; and UNaV 0.5 ± 0.2 to 0.6 ± 0.4, 0.6 ± 0.3, 0.5 ± 0.1 µmol·min⁻¹·g⁻¹. These findings as well as results from the experiments with
degraded ONOO⁻ solutions infusion do not indicate a time-dependent effect in the observed responses to low doses of ONOO⁻ administration. In our laboratory, time control experiments with intra-arterial saline infusions in anesthetized rats have been conducted in many previous studies (20–22) and demonstrate a stable preparation with insignificant changes in arterial pressure, renal hemodynamics, or excretory parameters for at least 4 h following completion of surgical procedures. Thus, the responses to ONOO⁻ administration reported in the present study are not due to time-dependent effects.

Protocol 2 Experiments

When the low dose of ONOO⁻ (10 μg·kg⁻¹·min⁻¹) was infused for a longer period of time (75 min) in rats, similar results were observed as with protocol 1. These results are summarized in Table 3. RBF values were measured by the renal flow probe in these experiments. Prolonged administration of ONOO⁻ at 10 μg·kg⁻¹·min⁻¹ significantly increased RBF by 8 ± 2% and GFR by 17 ± 5% and decreased RVR by 5 ± 1% and is similar to the changes that were observed with short-term infusion as in protocol 1. The changes in V, UNa⁺V, FEK⁺, and UNa⁺V were not statistically different. However, UNOXV increased and UISOV decreased significantly with this low-dose infusion of ONOO⁻ (Table 3).

DISCUSSION

In the present study, the acute effects of increasing doses of peroxynitrite on the kidney were investigated. Our most im-

Fig. 1. Changes in renal blood flow (RBF) in response to intra-arterial infusion of peroxynitrite (ONOO⁻) doses in rats. These responses are expressed as percent changes from the baseline value taken as 100%. *P < 0.05 vs. baseline values. #P < 0.05 vs. corresponding values in control rats.

Fig. 2. Changes in renal vascular resistance (RVR) in response to intra-arterial infusion of ONOO⁻ doses in rats. These responses are expressed as percent changes from the baseline value taken as 100%. *P < 0.05 vs. baseline values. #P < 0.05 vs. corresponding values in control rats.

Fig. 3. Changes in glomerular filtration rate (GFR) in response to intra-arterial infusion of ONOO⁻ doses in rats. These responses are expressed as percent changes from the baseline value taken as 100%. *P < 0.05 vs. baseline values. #P < 0.05 vs. corresponding values in control rats.

Fig. 4. Changes in urinary sodium excretion (UNa⁺V) in response to intra-arterial infusion of ONOO⁻ doses in rats. These responses are expressed as percent changes from the baseline value taken as 100%. *P < 0.05 vs. baseline values. #P < 0.05 vs. corresponding values in control rats.
important finding is that a low-dose (10 μg·kg⁻¹·min⁻¹) infusion of ONOO⁻, both in short term and in long term, resulted in increases in RBF and GFR, while the higher doses (20 and 40 μg·kg⁻¹·min⁻¹) progressively decreased these renal hemodynamic parameters (Figs. 1–3). While the responses to higher doses of ONOO⁻ could possibly be influenced by the cumulative effect of additional doses of ONOO⁻ in the same animal, this is unlikely because the biological half-life of ONOO⁻ is less than 1 s (31). Thus, recovery experiment with clearance collections after ONOO⁻ infusion was not carried out in the present study. However, we observed that when the infusion of ONOO⁻ was stopped and replaced by saline infusion at the end of long-term experiments (protocol 2), the RBF returned back to near control levels within 2–3 min indicating a short-half-life of the ONOO⁻ during infusion. Thus, the cumulative effect of additional doses of ONOO⁻, if any, would be very minimal and unlikely to influence the overall response pattern in these experiments.

The vasodilatory response to low-dose infusion of ONOO⁻ is associated with an increase in $U_{\text{NO}_x}$V and a decrease in $U_{\text{ISOV}}$V (Table 3). These data support our hypothesis that ONOO⁻ at low concentration produces a vasodilator effect. Although the concentration range of normal plasma ONOO⁻ has not been determined experimentally, it can be assumed that a low plasma level of ONOO⁻ exists in a physiological state since NO- $O_2^-$ interaction occurs normally in biological systems. As mentioned earlier, this low-dose infusion of ONOO⁻ achieved a plasma level that could be physiologically more relevant than concentrations achieved with higher doses representing a pathophysiologic condition. Interestingly, in the present experiments, it was observed that continuous ONOO⁻ infusion at low dose produced a renal vasodilatory response as opposed to a vasoconstrictor response induced by higher doses. The vasodilatory response to ONOO⁻ was abolished in rats pretreated with L-NAME, suggesting that the presence of NOS is required to facilitate this vasodilatory action. It is also interesting to note that infusion of ONOO⁻ doses markedly decreased GFR in NOS-inhibited rats but not in control rats indicating that intact NOS activity provides a protective function against the actions of ONOO⁻ in the kidney.

Peroxynitrite is formed by the spontaneous reaction of NO with $O_2^-$. This nonenzymatic reaction is a key element in understanding the contrasting roles of ONOO⁻ in physiology and in pathology. ONOO⁻ is a powerful oxidant and may contribute to various diseases affecting humans (31). As an oxidant radical, ONOO⁻ can directly induce cytotoxic effects such as sulfhydryl oxidation, protein tyrosine nitration, membrane lipid peroxidation, and DNA damage (6, 32, 33). These effects lead to dysfunction of important cellular processes causing disruption of cell signaling pathways, and the induction of cell death through both apoptosis (11) and necrosis (36). On the other hand, it has also been reported that ONOO⁻ administration causes vascular relaxation in several organ systems including coronary (25) and systemic arteries of dogs (19), rabbit aortic strips (29), and the hindlimb vascular bed of the rat (30). Systemic bolus administration of ONOO⁻ solution in rats (5) at doses 1–10 μmol/kg injected into the femoral vein also elicited vasorelaxant effects in the hindquarter and mesenteric vascular beds with a minimal change in renal vascular bed that could be related to its dose reaching the kidney. Furthermore, ONOO⁻ provided protection against ischemia-reperfusion injury in vivo (24) and did not impair vasodilator responses to acetylcholine or vasoconstrictor responses to ANG II (30). Indeed, under physiologic conditions, the production of ONOO⁻ should be low and oxidative damage minimized by endogenous antioxidant defenses (34). However, in pathologic conditions and with a moderate increase in ONOO⁻ formation over long periods of time, substantial oxidation, dysfunction, and destruction of host cellular constituents can occur (31).

Although previous studies provided evidence that the interaction between NO and $O_2^-$, and thus, the formation of ONOO⁻ is very important in providing a protective role in the kidney (13, 17, 20–22, 27, 28), the mechanisms associated with this protective effect are not yet understood. It is hypothesized that this renoprotective effect may be due to the conversion of ONOO⁻ to a NO donor-like substance, which would prolong the vasorelaxant properties of NO. In this regard, several hypotheses have been proposed, including increased formation of cGMP and nitrite by ONOO⁻, and the reverse reaction of ONOO⁻ has been reported to increase cGMP causing vascular relaxation by nitrosylating tissue and reducing glutathione or other thiols forming NO donors that subsequently release NO over prolonged periods of time (37). Another possible explanation is that the major end product of ONOO⁻ oxidation, the anion nitrite, would be reduced to NO (14). The results of our present study show increased urinary excretion of nitrite following ONOO⁻ administration in both control and L-NAME-treated rats. Circulating nitrite anion has been reported to have cytoprotective effects in pathologic conditions by acting as a direct vasodilator (10) or as a NO compound donor (12). It is reported that NOS, acting as a nitrite reductase, has the capacity to reduce nitrite to NO under anoxic condition in vitro (14). Although the activity of nitrite reductases is well-described in bacteria, some in vitro studies showed that certain mammalian enzymes may also have nitrite reductase activity in addition to their normal physiologic function (7, 9, 15, 18, 26). The present findings that the vasodilatory response to ONOO⁻ was abolished in rats pretreated with L-NAME allow us to hypothesize that NOS may act as nitrite reductase involved in the reduction of ONOO⁻ to NO. To demonstrate the in vivo activity of nitrite, a recent study showed that infusions of sodium nitrite into the human circulation produced significant vasodilation that appeared to be mediated by a nitrite reductase activity of deoxygated hemoglobin (8). An alternative explanation for the vasodilatory effects of ONOO⁻ is the reverse reaction, a nonenzymatic
reaction that causes the conversion of ONOO− to NO and O2−. This reaction is well-described in a recent review by Pacher et al. (31), showing that the degradation of both NO and O2− produced during the reverse reaction yields nitrite as the most important end product. Although the reverse reaction is much slower than the forward reaction, it appears to be significant in physiologic conditions. It was reported that inhibition of NOS enhances endogenous O2− activity (28). The finding that UISOV increased after l-NAME administration in the present study also indicates an increase in endogenous O2− level. In this regard, it is possible that the absence of a vasodilator action of ONOO− in l-NAME-treated rats could be due to enhanced O2− activity that inactivates NO and prevents its vasodilator action. Further experiments both in vivo as well as in vitro will be required to test the involvement of this putative mechanism(s) in the renal vasodilator action of ONOO−.

In the present study, infusion of ONOO− doses did not produce appreciable changes in V or UISOV in control rats. However, variable increases in V and UISOV were noted in response to ONOO− doses in l-NAME-treated rats. It seems interesting if we consider the fact that RBF was markedly decreased in that group of NOS-inhibited rats. Intra-arterial administration of sodium nitrate doses also showed variable increases in UISOV in NOS-inhibited dogs (16). The exact mechanism involved in such NOS-dependant or -independent actions of ONOO− is not yet clear. However, results from an earlier in vitro study (38) indicate that at a nanomolar concentration, ANG II inhibits Na+−K+−ATPase activity in rat proximal tubules and such inhibition is mediated by ONOO− formation. These data suggest that ONOO− may be involved in the diuretic and natriuretic responses to a high-dose administration of ANG II. It is possible that an amplification of ANG II actions following l-NAME administration (2) alters the reactivity of ONOO− on the renal tubules which results in such variable increases in UISOV. Further comprehensive studies are needed to investigate the direct action of ONOO− on renal tubular reabsorptive function.

In conclusion, we showed in rats that ONOO− at low infusion rate produces renal vasodilation, but higher infusion rates cause vasoconstriction. In addition, infusion of ONOO− in rats pretreated with l-NAME does not cause vasodilatory effects but rather produces a vasoconstrictor response. These findings indicate that intact NOS activity provides a protective function against the actions of ONOO− in the kidney.

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