Renal Nerves Do Not Mediate Vasoconstrictor Responses to Acute Nitric Oxide Synthesis Inhibition in Conscious Rats

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Abstract. Nitric oxide is a physiologically important peripheral and renal vasodilator. The studies presented here were conducted in the conscious, chronically catheterized, unstressed rat to investigate whether NO interacts with renal efferent sympathetic nerve activity in control of blood pressure, renal vascular resistance, and sodium excretion. Renal clearance studies were conducted in normal rats with innervated kidneys and in a separate group of rats with chronic, bilateral renal denervation. Acute systemic inhibition of NO synthesis with n-nitro L-arginine methyl ester (L-NAME) leads to hypertension, renal vasoconstriction, and natriuresis in rats with intact renal nerves. Chronic renal denervation does not diminish the pressor and renal vasoconstrictor response to NO synthesis inhibition, although the natriuretic response is prevented. Stimulation of renal NO synthesis with the substrate L-arginine produces selective renal vasodilation and a marked osmotic diuresis in the innervated kidney. Renal denervation has little impact on the responses to L-arginine. These studies suggest that in the normal, conscious, chronically catheterized rat in which the sympathetic nervous system is operating at basal levels, renal nerve activity does not contribute to the pressor or renal vasoconstrictor response to NO inhibition or the renal vasodilator response to NO stimulation. These observations contrast with earlier observations made under conditions of stress-induced activation of renal nerve activity. (J Am Soc Nephrol 8: 887–892, 1997)

Nitric oxide is tonically released by the activity of the constitutive endothelial and neuronal NO synthases (NOS) (1). Nitric oxide is vasodilatory, and tonically produced NO maintains both low blood pressure (BP) and high renal blood flow by causing systemic and renal vasodilation (1,2). When NO synthesis is acutely inhibited by administration of substituted L-arginine analogs, dose-dependent pressor and renal vasoconstrictor responses occur (2). The vasoconstriction secondary to acute NO synthesis inhibition (NOSI) is a result of the withdrawal of vasodilatory NO and the amplification of any vasoconstrictor systems that are operating. The contribution of the various vasoconstrictors to the maintenance of peripheral and renal vascular tone varies in response to many factors, including the experimental preparation utilized (which determines the level of activation of these systems).

The sympathetic nervous system (SNS) is tonically active and provides much of the short-term, physiologic regulation of vascular tone. Some studies suggest that central and/or peripheral sympathetic activation are partly responsible for the pressor and renal vasoconstrictor responses to acute NOSI (3–5). A specific role for renal efferent nerve activity in mediating the vasoconstrictor responses to both acute and chronic NOSI has been suggested (6–8). In addition, the renal vasodilatory response to L-arginine administration, thought to be mediated via stimulation of renal NO production, is abrogated by renal denervation. This suggests that a reduction in tonic efferent renal sympathetic nerve activity contributes to the renal vasodilatory response to L-arginine (9). An additional factor that further complicates the response to acute NOSI is that the abrupt rise in BP should lead to a baroreflex-mediated reduction in sympathetic tone.

In the studies presented here, we specifically evaluated the role of renal nerve activity on the systemic and renal responses to acute NOSI and acute L-arginine infusion. Studies were performed in the conscious chronically catheterized rat, a preparation in which the activity of the SNS (and other vasoconstrictor systems) is low (normal). Two groups of conscious rats were studied; in one group the renal nerves were intact, and the other group was subjected to chronic bilateral renal denervation. The overall goal was to investigate interactions between NO and renal nerve activity in a setting in which renal nerve activity was at the normal low value in the conscious chronically catheterized rat with innervated kidneys.

Materials and Methods

Studies were conducted on 18 male Sprague-Dawley rats, aged 3 to 5 months, obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN). In all rats, a preliminary surgery was conducted, in which catheters were placed in the left femoral artery and vein and in the urinary bladder. In seven of the rats, both kidneys were denervated prior to vascular and bladder catheterization, using low-power surgical microscopy. Bilateral renal denervation was carried out via an abdominal midline incision. All visible nerves supplying the kidney and around the renal artery, renal vein, and ureters were cut; these structures were then painted with a 10% phenol solution, which was rinsed with sterile 0.9% NaCl after approximately 5 min. All surgery
was conducted while the rats were under general anesthesia (using a short-acting barbiturate). At the end of the surgery, vascular catheters and bladder catheters were primed and plugged, and rats were returned to individual cages. Full sterile technique was used throughout the procedure. Details of this chronic catheterization method have previously been published (2,10–13). All rats were allowed free access to rat chow (approximately 24% protein and 0.4% Na) and drinking water and were handled and trained to accustom them to the activity in the laboratory. A period of 7 to 10 days elapsed between surgery and the acute experiments. When more than one study was conducted on the same animal, at least two days’ rest was allowed between experiments.

Renal function studies were conducted as follows: Rats were placed in a restraining cage and the arterial catheter was connected to a pressure transducer and recorder for BP measurement and occasional sampling of arterial blood. An intravenous infusion of 3H inulin (2 to 5 μCi/ml) and paraaminohippuric acid (PAH 1%) was given in 0.9% NaCl at 5 μl/min per 100 g rat body weight. The bladder pin was removed for collection of urine, and a tube with side arm was attached to the bladder catheter for collection of urine. After an 80-min equilibration period, two 20-min control urine collections were made with mid-point arterial blood samples. The bladder catheter was flushed with air 2 min before the end of each urine collection period to ensure the complete collection of urine. Mid-point blood samples (approximately 150 μl) were centrifuged, the plasma was removed for analysis, and the red cells were reconstituted with sterile 0.9% NaCl and restored to the rat.

After completion of the control measurements, one of the following experiments was conducted: In the first series of experiments, rats in groups 1 and 2 received the NO substrate L-arginine (L-arg; 300 mg/kg iv bolus, 50 mg/kg per min) for 50 min. L-Arg was added to the inulin and PAH solution used in the control experiment in a concentration of 1 g/ml, and two 20-min clearance measurements were made 10 to 50 min into the L-arg infusion. In the second series of experiments, rats in groups 3 and 4 received the NO synthesis inhibitor n-nitro L-arginine methyl ester (L-NAME; 10 mg/kg iv bolus) and two 20-min repeat clearance measurements were made 10 min after administration of the drug. At the end of the final clearance period, red blood cells were reconstituted and restored to the rat. Vascular and bladder catheters were primed and plugged, and rats were returned to their home cages, after which they were either used for additional experiments or euthanized. Both series of experiments were performed in rats with innervated (INN) and denervated (DNX) kidneys.

The following analyses and calculations were made: Urine volume was measured gravimetrically, and urine was then analyzed for 3H inulin activity and concentrations of PAH, Na, and K. Blood samples were analyzed for hematocrit value (Hct), plasma 3H inulin activity, and PAH, Na, and K concentrations. These measurements allow calculation of GFR, renal plasma flow (RPF), renal vascular resistance (RVR), urine flow (V), urinary excretion rate of Na⁺ and K⁺ (U Na,V and U K,V, respectively), and fractional excretion of Na (FE Na). We have previously published details of these analyses and calculations (2,10–13).

When all experiments were completed, rats were euthanized and the bladder and kidneys were inspected to establish whether they were free of infection. Both kidneys were removed, weighed, frozen in liquid nitrogen, and later assayed for norepinephrine content as follows. Kidney tissue was homogenized in perchloric acid and then filtered, and the extracted solution containing norepinephrine was run on HPLC (flow rate = 80 μl/min on a 100 × 1 mm C18 column; Sepstik, Waters/Millipore, Milford, MA). Norepinephrine was present in the kidneys of all normal intact INN rats, and no norepinephrine peak was seen in any of the kidneys harvested from the DNX rats (see the Results section), thus assuring completeness of denervation. Details of this HPLC assay are available elsewhere (14).

Within-group analysis was by paired t test and between-group analysis was by unpaired t test on absolute values or percentage change from control. All data is shown as mean ± 1 standard error (SE), and P < 0.05 is considered to be statistically significant. All experiments were conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

Results

In the control baseline state, there were relatively few differences between INN and DNX rats (Table 1). Body weight was slightly higher in the INN than DNX groups (403 ± 13 versus 348 ± 15 g, P < 0.05 in group 1 versus 2, which subsequently received L-arg; and 407 ± 12 versus 350 ± 10 g, P < 0.005, in group 3 versus 4, which subsequently received L-NAME). The only other variable that was different in the baseline state between INN and DNX rats was that U K,V was lower in group 4 DNX versus group 3 INN (P < 0.005); however, U K,V was similar in groups 1 and 2, INN versus DNX. Thus, complete chronic bilateral renal DNX has no effect on baseline renal hemodynamics or electrolyte excretion in rats studied 1 to 2 wk after DNX.

As shown in Table 1, acute L-arginine infusion in group 1 rats with innervated kidneys produced a selective renal vasodilatation that led to increased RPF without change in GFR. There was no effect on BP. L-Arg infusion also produced large increases in V, U Na,V, FENa, and U K,V, presumably via a solute diuresis. We previously reported similar renal responses in the conscious chronically catheterized rat in response to acute L-arg infusion (2,13,15). DNX rats showed a similar pattern of hemodynamic and excretory response to L-arg, as shown in Table 1. The magnitude of the fall in RVR and rise in RPF (expressed as percentage change from control) were similar in INN and DNX rats in response to L-arg (Figure 1). DNX did attenuate the increase in V, U Na,V, U K,V, and FENa when expressed in absolute terms (P < 0.05, 0.005, 0.005, and 0.001, respectively), although the percentage change versus control in these variables was not different in INN versus DNX (see Figure 1 for U Na,V). Thus bilateral renal DNX does not alter the acute renal hemodynamic response to L-arg and only slightly attenuates the natriuretic/diuretic response.

In the second series of experiments, we investigated the effect of acute systemic NOSI with L-NAME. In group 3 rats with innervated kidneys, intravenous L-NAME produced a large rise in BP, renal vasoconstriction, and significant falls in GFR and RPF. A natriuretic and diuretic effect was seen without any change in U K,V (Table 1). These observations are similar to those that we previously reported in the normal, conscious, chronically catheterized rat (2,10,11). A pressor and renal vasoconstrictor response with falls in GFR and RPF was also seen with acute NOSI in Group 4 DNX rats. The increase in BP with acute L-NAME was greater in DNX versus INN rats both in absolute terms (Table 1; P < 0.01) and when expressed as a percentage change from control (Figure 1). The increase in RVR and falls in RPF and GFR with acute NOSI
Table 1. Summary of renal responses, in the conscious chronically catheterized rat with either bilateral renal denervation (DNX) or intact innervated (INN) kidneys

<table>
<thead>
<tr>
<th>Group</th>
<th>BP (mmHg)</th>
<th>RVR (mmHg/ml/min)</th>
<th>GFR (ml/min)</th>
<th>RPF (ml/min)</th>
<th>V (µl/min)</th>
<th>U_{Na}V (µeq/min)</th>
<th>FE_{Na} (%)</th>
<th>U_{K}V (µeq/min)</th>
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<tr>
<td><strong>Group 1 INN</strong></td>
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<tr>
<td>control (n = 10)</td>
<td>119 ± 2</td>
<td>6.1 ± 0.3</td>
<td>2.82 ± 0.12</td>
<td>10.9 ± 0.6</td>
<td>18.7 ± 2.0</td>
<td>2.16 ± 0.28</td>
<td>0.541 ± 0.063</td>
<td>2.45 ± 0.22</td>
</tr>
<tr>
<td>exp + L-arg</td>
<td>120 ± 3</td>
<td>4.6 ± 0.3</td>
<td>3.14 ± 0.18</td>
<td>15.3 ± 1.0</td>
<td>251.8 ± 14.9</td>
<td>26.20 ± 2.04</td>
<td>6.134 ± 0.453</td>
<td>7.95 ± 0.62</td>
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<tr>
<td>P value, control versus exp</td>
<td>NS</td>
<td>&lt;0.005</td>
<td>NS</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td><strong>Group 2 DNX</strong></td>
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<tr>
<td>control (n = 6)</td>
<td>119 ± 2</td>
<td>6.7 ± 0.4</td>
<td>2.43 ± 0.30</td>
<td>9.8 ± 0.6</td>
<td>17.7 ± 4.4</td>
<td>1.52 ± 0.52</td>
<td>0.442 ± 0.126</td>
<td>1.80 ± 0.14</td>
</tr>
<tr>
<td>exp + L-arg</td>
<td>121 ± 4</td>
<td>4.9 ± 0.4</td>
<td>3.28 ± 0.45</td>
<td>14.6 ± 1.5</td>
<td>173.3 ± 29.5</td>
<td>12.47 ± 3.15</td>
<td>2.835 ± 0.741</td>
<td>4.51 ± 0.58</td>
</tr>
<tr>
<td>P value, control versus exp</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
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<td><strong>Group 3 INN</strong></td>
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<tr>
<td>control (n = 8)</td>
<td>119 ± 2</td>
<td>5.5 ± 0.5</td>
<td>3.01 ± 0.08</td>
<td>12.6 ± 1.0</td>
<td>21.3 ± 4.0</td>
<td>2.40 ± 0.53</td>
<td>0.564 ± 0.114</td>
<td>2.62 ± 0.23</td>
</tr>
<tr>
<td>exp + L-NAME</td>
<td>154 ± 3</td>
<td>13.0 ± 0.8</td>
<td>2.40 ± 0.18</td>
<td>6.8 ± 0.4</td>
<td>67.2 ± 13.7</td>
<td>5.34 ± 1.25</td>
<td>1.629 ± 0.381</td>
<td>2.46 ± 0.21</td>
</tr>
<tr>
<td>P value, control versus exp</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>NS</td>
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<tr>
<td><strong>Group 4 DNX</strong></td>
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<tr>
<td>control (n = 7)</td>
<td>118 ± 1</td>
<td>6.4 ± 0.4</td>
<td>2.82 ± 0.14</td>
<td>10.7 ± 0.6</td>
<td>17.1 ± 1.8</td>
<td>1.30 ± 0.36</td>
<td>0.333 ± 0.082</td>
<td>1.62 ± 0.15</td>
</tr>
<tr>
<td>exp + L-NAME</td>
<td>166 ± 3</td>
<td>17.5 ± 1.1</td>
<td>2.06 ± 0.24</td>
<td>5.5 ± 0.4</td>
<td>32.9 ± 7.4</td>
<td>1.90 ± 0.68</td>
<td>0.617 ± 0.160</td>
<td>1.56 ± 0.27</td>
</tr>
<tr>
<td>P value, control versus exp</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</table>

*All rats were studied in an initial control period, and groups 1 and 2 then received L-arginine (L-arg, 300 mg/kg iv bolus; 50 mg/kg per min); groups 3 and 4 received acute high dose n-nitro-L-arginine methyl ester (l-NAME, 10 mg/kg, iv). BP, blood pressure; RVR, renal vascular resistance; GFR, glomerular filtration rate; RPF, renal plasma flow; V, urine flow; U_{Na}V, urinary excretion rate of Na; FE_{Na}, fractional excretion of Na; U_{K}V, urinary excretion rate of K; exp, experimental; NS, not statistically significant.*
were similar in Groups 3 and 4. In contrast, the diuretic and natriuretic response to acute NOSI was absent in rats with bilateral renal DNX (Table 1, Figure 1).

The norepinephrine content of the homogenized left and right kidney cortex of control INN kidneys was 379 ± 22 ng/g tissue. No norepinephrine peak was detected in left or right kidneys of any DNX rats and because the limit of detectability of this assay is approximately 30 ng/g tissue, we can assume that all of the kidneys in the DNX group were functionally denervated.

Discussion

The studies presented here confirm that acute systemic NOSI with L-NAME produces widespread vasoconstriction in the conscious chronically catheterized rat, leading to an increase in BP and RVR. Exactly how this vasoconstriction is mediated is not yet clear, and these studies are part of a series of experiments intended to gain insight into this question. The major findings in this study are that renal sympathetic nerve activity is not involved in mediating the BP or renal vascular responses to either acute NOSI or acute NO stimulation with L-arginine, whereas renal nerve activity is required for the natriuretic and diuretic response to acute NOSI.

L-NAME is a nonselective NOS; thus, the pressor and renal vasoconstrictor response to L-NAME seen here is the net result of inhibition of NO synthesized by all tonically active NOS. NO is tonically produced by vascular endothelial NOS (eNOS) and the widely distributed neuronal NOS (nNOS). We do not yet know the relative contribution of these various NOS isoforms in terms of control of total and regional vascular tone, nor do we know how NO interacts with other vasoactive control systems. Studies in genetically manipulated mice suggest that NO generated from the eNOS plays the primary role in control of vascular tone (16). In contrast, functional evidence suggests that NO generated from nNOS has the potential to control vascular tone by multiple mechanisms. For example, NO generated within the central nervous system inhibits central sympathetic outflow (5,17). Systemic administration of nonselective NOS inhibitors, such as L-NAME, produces nNOS inhibition within the central nervous system (18). Systemic NOSI increases renal sympathetic nerve activity (4,9), an effect prevented by spinal transection, suggesting that systemic NOSI causes a centrally mediated increase in sympathetic tone (4). In addition, nitroxidergic peripheral nerves function as a vasodilatory counterbalance to adrenergic vasoconstrictor nerves in a number of locations, including the renal arteries (19).

In addition to specific interactions between NO and the SNS, nonspecific interactions occur secondary to alterations in vascular tone/BP. For example, shear provides a major regulator for vascular endothelial NO release; thus any maneuver that alters vascular tone and, therefore, endothelial shear forces (including alterations in activity of the SNS) will effect NO release (1). The inhibition of sympathetic activity via the baroreceptor reflex, activated by an abrupt rise in BP, is an additional nonselective interaction. Based on this interaction, one would predict that a pressor dose of L-NAME should produce a widespread reduction in sympathetic discharge, including a fall in renal sympathetic nerve activity. In fact, a transient fall in renal sympathetic nerve activity is seen in anesthetized rats 1 to 2 min after they receive a pressor dose of NOSI; however, renal sympathetic nerve activity rapidly increases to supernormal values despite persistent hypertension (9). Vagotomy with sinoaortic deafferentation prevents the transient fall and potentiates the prolonged rise in renal sympathetic nerve activity during acute NOSI. This suggests that baroreceptor-mediated reductions in renal sympathetic nerve activity attenuate the stable increase in activity in the renal nerves after NOSI in anesthetized rats (9). How renal nerve activity responds to acute NOSI in the awake, unstressed rat is, however, not known; the findings of the study presented here indicate that renal nerve activity remains low (see below). This is in accord with recent studies in normal unstressed humans, suggesting that baroreceptor-mediated reductions in sympathetic tone predominate because acute NOSI suppresses muscle sympathetic nerve activity (20).
This study specifically investigates the role of the renal nerves in mediating either pressor and/or renal vasoconstrictor responses to acute systemic NOSI, because several previous studies have claimed that increased renal efferent sympathetic nerve activity mediates the renal vasoconstriction and, possibly, the pressor response to acute NOSI. Preliminary observations by Granger and colleagues in the dog suggest that renal DNX attenuates the increased RVR with acute NOSI (6). A series of studies by Gabbai, Vallon, Thomson, Blantz, and colleagues claimed that renal denervation attenuates the glomerular hemodynamic responses to acute systemic NOSI in the anesthetized rat (8,21). These workers further conclude that the interaction between NO and the SNS involves α2 adrenergic-receptors, which may be interacting with the angiotensin II system (21,22). However, scrutiny of the data in these studies reveals that the increase in afferent and efferent arteriolar resistances with acute systemic NOSI is similar in rats with innervated and chronically denervated kidneys (21,22). Chronic NOSI with L-NAME administration for several weeks produces a dose-dependent model of systemic hypertension with renal vasoconstriction and eventual development of glomerular injury (23,24). There is some evidence that chronic bilateral renal denervation in the rat attenuates the glomerular injury (6) and hypertension (25), although this is not true for the dog (26).

The observations we report here are those predicted in a normal animal in which renal nerve activity is low and not controlling renal vascular tone under basal conditions and in which renal nerve activity is not activated by acute NOSI. When renal sympathetic nerve activity is high, secondary to surgical and/or emotional stress, heavy exercise, etc., renal nerve activity is controlling renal vascular tone. In this situation, withdrawal of vasodilatory NO could potentiate renal vasoconstriction because of the enhanced renal nerve activity. Increased renal nerve activity as a result of the preparation may underlie some of the variability in the literature; for example, the renal vasodilatory response to the NO substrate L-arg can be prevented by prior renal denervation in the conscious rabbit (9). These studies suggest that, at least in the conscious rabbit, L-arg vasodilates the kidney by suppression of tonic renal efferent sympathetic nerve activity (9), which in turn implies that RVR is controlled by the SNS, a condition consistent with elevated stress levels. This is clearly not the case in the conscious chronically catheterized rat because in the study presented here, renal denervation did not attenuate the renal vasodilatory response to L-arg. Therefore, these studies confirm that alterations in renal nerve activity are not essential for expression of the renal vascular responses to acute NO synthesis inhibition or stimulation with L-arg.

Acute systemic NOSI, when accompanied by an abrupt rise in BP, leads to significant increases in urine flow and sodium excretion rates, an effect that we previously suggested might be a result of a pressure natriuresis (2). The natriuretic and diuretic response to L-arg infusion is primarily the result of a nonspecific osmotic diuretic effect, because a similar response is seen with the inactive D-arginine (15). In the study presented here, chronic renal DNX slightly attenuates diuretic/natriuretic responses to L-arg and is particularly effective in inhibiting the diuretic and natriuretic responses to acute systemic NOSI. A similar observation was reported by Khrabi in the spontaneously hypertensive rat (SHR). The SHR has hyperactive renal nerve activity, which is anti-natriuretic through increased re-absorption in the proximal tubule. The natriuretic response to acute systemic NOSI in the SHR is largely prevented by renal denervation, whereas in the Wistar-Kyoto normotensive rat, renal denervation does not influence the magnitude of the natriuretic/diuretic response (27). Exactly how renal nerve activity contributes to the natriuretic/diuretic response to acute NOSI and acute L-arg infusion in the conscious normotensive Sprague-Dawley rat in the study presented here is unclear.

In summary, the tonic low level of renal nerve activity present in the conscious, unstressed, normotensive Sprague-Dawley rat does play some role in the natriuretic/diuretic responses to acute NOSI or L-arg infusion. However, renal nerve activity plays no role in the expression of the renal vasoconstriction in response to NOSI or renal vasodilation in response to NO stimulation with L-arg in this conscious rat preparation.

Acknowledgments
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References


