Abstract

Recent studies have indicated that chronic administration of \(N^\text{\textsuperscript{\text}O}\)-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) synthesis, produces marked hypertension. Although the mechanism of this form of hypertension is not well understood, several studies have demonstrated that sympathetic nerve activity is at least acutely elevated after L-NAME administration. To evaluate the potential role of the renal sympathetic nerves in L-NAME-induced hypertension, we compared the blood pressure response to L-NAME in four groups of Sprague-Dawley rats \((n=8\) each): (1) sham-operated vehicle-treated, (2) sham-operated L-NAME-treated, (3) denervated vehicle-treated, and (4) denervated L-NAME-treated. After renal denervation or sham surgery, L-NAME was added to the drinking water (70 mg/L 100 mL) for 4 weeks, and arterial pressure was measured weekly by the tail-cuff method. L-NAME treatment caused a progressive increase in arterial pressure in sham-operated rats, rising to 154±6 mm Hg by week 4 of treatment compared with 115±2 mm Hg in the vehicle-treated sham-operated group \((P<.005)\). In contrast, the development of hypertension was significantly delayed and attenuated in renal-denervated rats treated with L-NAME. The results of our study suggest that L-NAME-induced hypertension may be partly mediated by or is at least dependent on the integrity of the renal nerves. (Hypertension. 1994;23[part 2]:971-975.)

Key Words

- blood pressure
- endothelium-derived relaxing factor
- nitric oxide
- sympathetic nervous system
- denervation
- microalbuminuria
- natriuresis

Nitric oxide (NO) is now known to be an important participant in a variety of physiological processes, including several that influence arterial pressure.\(^1\) Acute administration of substituted arginine analogues that inhibit NO synthesis, including \(N^\text{\textsuperscript{\text}O}\)-nitro-L-arginine methyl ester (L-NAME), results in a prompt increase in arterial pressure.\(^2\) The immediate increase in arterial pressure may be principally due to an increased vascular smooth muscle tone as a consequence of decreased endothelial synthesis of NO. Increases in sympathetic nerve activity have been described after acute NO synthesis inhibition,\(^3\)\(^-\)\(^6\) suggesting that neurogenic mechanisms may also contribute to the acute increase in arterial pressure.

Several laboratories have recently reported that continued administration of inhibitors of NO synthesis induces a sustained hypertension.\(^7\)\(^-\)\(^9\) Although a precise mechanism by which continued NO synthesis inhibition may induce chronic hypertension remains to be identified, renal control of fluid and electrolyte balance is thought to play a dominant role in the long-term control of arterial pressure in both normal and pathophysiologic states.\(^10\)\(^-\)\(^11\) Renal sympathetic nerve activity is known to be increased at least acutely after administration of NO synthesis inhibitor,\(^4\)\(^-\)\(^6\) and activation of the renal sympathetic nerves is known to inhibit renal sodium excretion and promote renal renin secretion.\(^12\) Thus, we hypothesized that chronic L-NAME-induced hypertension may be, in part, the result of a sustained activation of the renal sympathetic nerves and the resultant resetting of renal fluid and electrolyte balance to higher arterial pressures. To test this hypothesis, we compared the course of arterial pressure in renal-denervated and sham-operated rats during 4 weeks of oral administration of L-NAME or vehicle.

Methods

Animals

To examine the effects of renal denervation during the administration of L-NAME, a total of 32 male Sprague-Dawley rats (Japan SLC Inc) were assigned randomly to one of four groups: (1) sham-operated vehicle-treated (S/V), (2) sham-operated L-NAME-treated (S/L), (3) renal-denervated vehicle-treated (D/V), and (4) renal-denervated L-NAME-treated (D/L) rats. Each of the four groups consisted of 8 age- and weight-matched rats. The procedures and protocol of the study were in accordance with our institutional guidelines. Throughout the study, the rats were housed in a room that was kept at constant temperature (25±1°C) and humidity (60±5%) and was lighted automatically from 7 AM to 7 PM. Rats were provided with free access to water and rat chow (CE-2, Clea Japan) that provided 110 mmol of sodium and 280 mmol of potassium per kilogram of chow.

Renal Denervation and Sham Surgery

Rats were anesthetized with pentobarbital sodium (50 mg/kg IP). Renal denervation was done by stripping the nervous and connective tissue passing to and along the course of the renal artery and vein of both kidneys and painting these vessels with a solution of 10% phenol in ethanol through a midline abdominal incision. Sham-operated control rats received only a midline incision, but the renal nerves were left intact.\(^13\) Renal denervation was verified at the end of the experiment by analysis of the renal norepinephrine content.

Experimental Protocol

Experiments were begun 3 to 5 days after renal denervation or sham surgery. The experiment consisted of 1 control week followed by 5 weeks of oral administration of L-NAME (0.7 g/L drinking water) or vehicle. In the control week and

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subsequent 4 weeks of treatment with L-NAME or vehicle, arterial pressures were estimated weekly in conscious restrained rats by the tail-cuff method by use of an electrosphygmomanometer (PS-100, Riken Kihatsu). On the last day of each week, rats were weighed and housed in individual metabolic cages to measure water intake, urine volume, and urinary excretions of sodium and albumin over 24 hours on the last day of each week.

In week 5, the rats were catheterized to verify the blood pressure level by direct measurement. Rats were anesthetized with pentobarbital (50 mg/kg), and a catheter (PE10 connected to PE50) was passed into the lower abdominal aorta via the right femoral artery. The catheter was then tunneled subcutaneously, exteriorized between the scapulae, and filled with saline containing sodium heparin (500 U/mL). After a 2-day recovery period, mean arterial pressure (MAP) was monitored (model TP-101T, Nihon Koden) for 1 hour while the conscious rats rested on wood shavings in a Plexiglas cage. Reported pressures represent the average MAP during the last 15 minutes of the recording period.

After direct measurement of MAP, blood was withdrawn for analysis of plasma renin activity (PRA). The animals were then killed, and their left kidneys were immediately removed and frozen in liquid nitrogen for subsequent analysis of norepinephrine content.

Chemical Analyses

Plasma was separated from whole blood samples immediately after sampling by centrifugation. Urine sodium was determined by flame photometer (No. 710, Hitachi). As a quantitative estimate of glomerular damage, urinary albumin concentration was determined by an enzyme-linked immunosorbent assay kit (Nephrat, Exocell Inc). The urinary albumin excretion rate was calculated as the product of the daily urinary volume and urinary albumin concentration.

The frozen kidneys were rapidly minced and placed in ice-cold 0.3N perchloric acid (total volume, 12 mL). The kidneys were homogenized, the homogenate was centrifuged at 20000g for 20 minutes, and the supernatant was stored at −80°C until analyzed. Renal norepinephrine content was determined by high-performance liquid chromatography with electrochemical detection (HLC-725CA, Tohso). PRA was determined with a radioimmunoassay kit (Gamma Coat, Bayer).

Statistical Analyses

Experimental groups were compared by analysis of variance and, when appropriate, with Scheffe's test for multiple comparisons. All data are expressed as mean±SEM unless otherwise indicated.

Results

Effects on Metabolic Variables

The time courses of metabolic effects of L-NAME treatment and renal denervation are summarized in Fig 1. Although all groups of rats gained weight during the course of the study, the weight gain of L-NAME-treated rats tended to level off by the last week (S/V, 363±4; D/V, 351±11; S/L, 334±5; and D/L, 341±9 g). Although there was considerable variation between individual rats and groups, urinary sodium and water excretion appeared to be attenuated in L-NAME-treated sham-operated animals (Fig 1). The rate of urinary albumin excretion was similar in each of the four groups during the first week (Fig 1). In weeks 2 through 4, L-NAME–treated sham-operated animals also stood out as having a greatly increased urinary albumin excretion. Relatively little change was evident in the other
three groups. At its peak in week 3, the urinary albumin excretion of L-NAME-treated sham-operated rats was significantly greater than all three control groups, being 2.5, 3.9, and 2.6 times greater than those of the sham-operated vehicle-treated, renal-denervated vehicle-treated, and renal-denervated L-NAME-treated groups, respectively (S/V, 0.22±0.04; D/V, 0.14±0.02; S/L, 0.55±0.01; and D/L, 0.21±0.02 mg/24 hours).

**Effect on Arterial Pressure**

Tail-cuff pressure and heart rate were not different among the four groups during the baseline measurement period. After 1 week, tail-cuff pressure was significantly increased in sham-operated L-NAME-treated rats compared with all other groups (Fig 2; S/V, 110±2; D/V, 101±2; S/L, 138±2; and D/L, 112±2 mm Hg, P<.0001). Tail-cuff pressure reached 154±6 mm Hg by week 4 in L-NAME-treated compared with 115±2 mm Hg in sham-operated rats treated with vehicle (P<.005). In contrast, tail-cuff pressure remained at control levels during weeks 1 and 2 in renal-denervated rats treated with L-NAME and was not significantly elevated until week 4 (Fig 2). That is, renal denervation appeared to delay and/or attenuate L-NAME-induced hypertension. Heart rate fell during the first week of the experiment in both L-NAME–treated groups (Fig 2; S/V, 329±9; D/V, 352±2; S/L, 290±7; and D/L, 285±9 beats per minute, P=.0001) and remained low throughout the experimental period.

MAPs recorded directly from arterial catheters in conscious rats in week 5 are shown in the Table. MAP was markedly elevated in both L-NAME–treated groups compared with their respective vehicle-treated control groups. In contrast with earlier tail-cuff measurements of arterial pressure, the MAP of sham-operated L-NAME–treated rats was only marginally elevated above that of renal-denervated rats treated with L-NAME. This difference was not statistically significant. Direct measurements of the MAP correlated significantly with arterial pressures measured by the tail-cuff method in week 4 (r=.83, P=.0001).

**PRA and Renal Norepinephrine Content**

The PRA and renal norepinephrine content of animals killed at week 5 after surgery are summarized in the Table. Although PRA appeared to be somewhat elevated in L-NAME–treated sham-operated animals relative to other groups, there were no statistical differences among the four groups.

In L-NAME– and vehicle-treated groups, renal denervation significantly reduced the renal norepinephrine content by 93% and 94%, respectively, relative to the levels in sham-operated controls (Table). There were no significant differences between L-NAME– and vehicle-treated groups.

### Mean Arterial Pressure, Renal Norepinephrine Contents, and Plasma Renin Activity at the End of Experiments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>MAP, mm Hg</th>
<th>Renal NE, ng/g tissue</th>
<th>PRA, ng Al·ml⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham vehicle</td>
<td>8</td>
<td>107±4</td>
<td>162±13</td>
<td>4.9±1.0</td>
</tr>
<tr>
<td>Sham L-NAME</td>
<td>8</td>
<td>151±5†</td>
<td>175±16</td>
<td>6.1±1.5</td>
</tr>
<tr>
<td>Denervated vehicle</td>
<td>8</td>
<td>109±5</td>
<td>11±3§</td>
<td>3.6±1.0</td>
</tr>
<tr>
<td>Denervated L-NAME</td>
<td>8</td>
<td>147±5†</td>
<td>8±1§</td>
<td>4.0±0.9</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; NE, norepinephrine; PRA, plasma renin activity; Al, angiotensin I; and L-NAME, N⁴-nitro-L-arginine methyl ester.

*P<.05 compared with vehicle-treated sham-operated group; †P<.05 compared with measurements in vehicle-treated denervated group; §P<.05 compared with measurements in L-NAME–treated sham-operated group.
intravenous administration of N\textsuperscript{G}-methyl-L-arginine, sympathetic nerve activity. Sakuma et al\textsuperscript{4} showed that reports of the effects of NO synthesis inhibitors on renal synthesis inhibitors. Such a role was in response to hypertension caused by chronic administration of NO \textsuperscript{L}-NAME-treated rats.

The mechanism responsible for the eventual increase in arterial pressure in renal-denervated rats treated with L-NAME is not clear. Since the renal sympathetic innervation may begin to recover from renal denervation within as little as 4 weeks in rats,\textsuperscript{22} repeated surgeries may be required to ensure sustained renal denervation in long experiments.\textsuperscript{32} However, our protocol was complete within 6 weeks of renal denervation or sham surgery, and renal norepinephrine levels were less than 7% of control levels by the end of the experiment (Table), suggesting that significant reinnervation had not yet occurred. It is possible that mechanisms unrelated to the renal nerves may be responsible for the eventual rise in arterial pressure in renal-denervated L-NAME--treated rats.

The significance of our present results is that they support a role for the sympathetic nervous system in the hypertension caused by chronic administration of NO synthesis inhibitors. Such a role was in response to reports of the effects of NO synthesis inhibitors on renal sympathetic nerve activity. Sakuma et al\textsuperscript{4} showed that intravenous administration of N\textsuperscript{G}-methyl-L-arginine, also an inhibitor of NO synthesis, increased renal sympathetic nerve activity. This effect was abolished by spinal section but not by vagotomy or sinoaortic baroreceptor denervation. In addition, injection of NO synthesis inhibitors intracisternally\textsuperscript{5} and directly into the nucleus tractus solitarius\textsuperscript{6} and rostral ventrolateral medulla\textsuperscript{24} has also been shown to increase arterial pressure and renal sympathetic nerve activity. These results suggest that NO may be involved in the brain stem regulation of sympathetic nerve activity and raise the possibility that NO inhibitors may influence the renal control of sodium excretion and arterial blood pressure through this mechanism.

Although the rationale for renal denervation has generally been to interrupt sympathetic nerve activity directed to the kidney, denervation of the renal plexus also deprives the kidney of its sensory innervation. Selective renal afferent denervation may have remarkably widespread effects on the sympathetic system, including reducing central sympathetic neurotransmitter stores.\textsuperscript{23,24} Selective afferent renal denervation has been reported to attenuate hypertension in the one-kidney, one clip model and to cause a small reduction in blood pressure in the spontaneously hypertensive rat.\textsuperscript{22} However, negative results were reported for selective afferent denervation during the development of hypertension in the spontaneously hypertensive rat, during renal wrap hypertension, and during hypertension caused by infusion of norepinephrine into the renal artery.\textsuperscript{22} Since our present experiments were not specifically designed to distinguish between the effects of afferent and efferent renal denervation, we cannot discount an influence of the afferent renal nerves on our results.

We attempted to verify the ability of renal denervation to attenuate L-NAME--induced hypertension, as determined by the tail-cuff technique in weeks 0 to 4, by direct measurement of MAP in week 5. Although pressures measured by the tail-cuff method at week 4 of the experiment strongly correlated with direct catheter measurements of MAP in week 5, the MAPs of L-NAME--treated renal-denervated and sham-operated animals were rather similar and not statistically different. Since tail-cuff pressures of renal-denervated L-NAME--treated rats were increasing in weeks 3 and 4 of the experiment, further increases in the arterial pressure of renal-denervated L-NAME--treated rats may account for the similarity of blood pressures of renal-denervated and sham-operated rats in week 5. Subsequent experiments in a small number of animals (n=3 per group) have confirmed, by direct measurement of MAP, that the arterial pressures of renal-denervated and sham-operated animals are indeed different in the first 2 weeks of L-NAME treatment (S/L, 124±6; D/L, 105±5 mm Hg). Thus, our results suggest that renal denervation may be particularly effective in delaying the onset of L-NAME--induced hypertension.

In conclusion, our results demonstrate that bilateral renal denervation delayed and possibly attenuated hypertension induced by long-term administration of L-NAME, an inhibitor of NO synthesis. The results of our study suggest that much of the hypertension induced by chronic NO synthesis inhibition is mediated by or at least dependent on the integrity of the renal nerves.
Acknowledgments
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