Long-term Cardiovascular Role of Nitric Oxide in Conscious Rats

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Abstract The goal of this study was to determine the arterial pressure and renal excretory responses to a continuous intravenous infusion of L-NAME in conscious rats. Studies were conducted in six groups of Sprague-Dawley rats with indwelling arterial and venous catheters over periods lasting 12 to 26 days. In the first group of rats, L-NAME infusion for 9 days caused a sustained increase in arterial pressure, and on the ninth day arterial pressure was increased 29 mm Hg. Infusion of L-NAME at the higher dose of 37 nmol/kg per minute for 9 days caused no greater increase in arterial pressure than the lower dose. Sodium and volume balances and phenylephrine pressor sensitivity were unchanged during L-NAME administration at 7.4 nmol/kg per minute; plasma renin activity increased 2.5-fold, but the vasodepressor and vasodilator responses to acetylcholine and bradykinin were unchanged. Arterial pressure remained significantly increased 7 days after L-NAME was stopped, but in another group of rats, intravenous L-arginine infusion caused arterial pressure to return to control within 1 day. This same dose of L-arginine was administered for 7 days intravenously, and neither arterial pressure nor sodium balance changed. In other groups of rats, L-arginine was administered in conjunction with L-NAME; this prevented any change in arterial pressure, whereas D-arginine did not. In conclusion, the data suggest that continuous intravenous infusion of L-NAME causes sustained increases in arterial pressure in conscious rats without any sodium or water retention. The hypertension is accompanied by increases in plasma renin activity and can be prevented with intravenous L-arginine administration. (Hypertension. 1994;23:185-194.)

Key Words • endothelium • vasodilation • hypertension • arginine

Methods

Animal Preparation, Experimental Measurements, and Instrumentation

Long-term experiments were conducted in 34 conscious male Sprague-Dawley rats with an average body weight of 412±11 g (Harlan Sprague Dawley Inc, Indianapolis, Ind). The project was approved by the local Institutional Animal Committee. Aseptic technique was used throughout all surgical procedures; body temperature was maintained at approximately 37°C with a heating pad. With animals under sodium pentobarbital anesthesia (0.2 mmol/kg IP), a laparotomy was performed, and a nonocclusive, polyvinyl catheter was inserted into the abdominal aorta, caudal to the kidneys; the insertion point was sealed with cyanoacrylate adhesive. In addition, an inferior vena caval catheter was inserted through the femoral vein, and a superior vena caval catheter was inserted through the jugular vein. All catheters were routed subcutaneously to the scapular region where they were exteriorized. Prophylactic antibiotics (Mezlin, 0.05 mmol/d, Miles Inc, West Haven, Conn) and penicillin G (25 000 U/d, Bristol-Myers Squibb Co, Princeton, NJ) were given throughout the experiment in an intravenous infusion of 18 mL isotonic NaCl per day. The rats were allowed to recover from surgery and were placed in individual metabolic cages in a quiet, air-conditioned room with a 12-hour light/dark cycle.

The arterial and femoral catheters were connected to a dual-channel infusion swivel (Instech Laboratories Inc, Plymouth Meeting, Pa) mounted above the cage and were protected by a stainless steel spring that also served as a tethering device. Saline solutions were infused intravenously with a syringe pump (Harvard Apparatus, South Natick, Mass) through a filter (Cathivex, 0.22 internal diameter, Millipore Corp, Bedford, Mass). The arterial catheter was filled with a heparin solution (1000 USP U/mL) and connected via the swivel to a pressure transducer (Cobe, Lakewood, Colo) mounted on the cage exterior. Pulsatile arterial pressure
signals from a polygraph (model 7D, Grass Instrument Co, Quincy, Mass) were sent to a digital computer through an analog-to-digital converter. The analog signal from the polygraph was sampled at 500 Hz for 4 seconds of each minute over a 21-hour period each day. From these data samples, arterial pressure and heart rate were determined each minute and were stored on a computer disk.

Total sodium intake throughout the experiment was maintained at 2.8 mmol/d by continuous intravenous infusion of 18 mL of sterile 0.9% saline per day and low-sodium chow (0.006 mmol sodium/g, Teklad Premier Laboratory Diets, Madison, WIs). This infusion was begun immediately after placement of the rat in the metabolic cage. At least 1 week was allowed for recovery and acclimation to the animal room before control measurements were begun. In addition, food and water intake and urine volume were monitored daily during the recovery from surgery, and no experimental measurements were begun until these variables had stabilized.

Plasma and urine sodium and potassium concentrations were measured by flame photometry, and plasma renin activity was measured by radioimmunoassay. Water intake and urine volume were measured daily. Volume balance was calculated by adding the water intake by drinking and the volume of solutions infused and subtracting urine volume. However, some water content in the food adds to volume balance, and fecal and insensitive loss of water subtracts from the balance; the sum of all these factors is equal to the volume balance correction. Volume balance correction was determined by taking the difference between total volume intake and excretion and averaging this over the 7-day control period. Then daily volume balances were adjusted by subtracting the volume balance correction, which gave a more accurate estimate of the true balance. Sodium balances were calculated in a similar way.

Studies on the vasodilator and vasoconstrictor effects of NO agonists were conducted in an additional 14 male Sprague-Dawley rats. In 6 of these rats, a jugular vein catheter was implanted aseptically as previously described for a 6-day intravenous infusion of L-NAME at 7.4 nmol/kg per minute. After the L-NAME infusion, these 6 rats plus an additional 8 rats in the control group were anesthetized with sodium pentobarbital. Then, a polyvinyl catheter was inserted into the carotid artery for measurement of arterial pressure, a nonocclusive polyvinyl catheter was inserted into the abdominal aorta as previously described for the intraarterial infusion of solutions, and an electromagnetic flow probe (1.0 mm inside diameter, Zepeda Instruments, Seattle, Wash) was placed on the iliac artery. Also, the control group had a polyvinyl catheter inserted into the jugular vein for infusion of solutions. During this acute protocol, rectal temperatures were controlled at 37°C, and all rats received intra-arterial and intravenous doses of acetylcholine, bradykinin, and sodium nitroprusside both before and 20 minutes after the intravenous infusion of 15.7 μmol/kg of meclofenamate sodium that was dissolved in 5 mmol/L Na₂CO₃. The dosages of these substances are reported in Table 2. Pressure and flow data were sampled by a digital computer through an analog-to-digital converter at 200 Hz continuously for approximately 2 to 3 minutes after the injections. From these data, the following were determined: the maximum decrease in mean arterial pressure, the maximum increase in iliac blood flow, the area under the iliac flow-time curve, and the changes in iliac arterial resistance.

Drugs Used

Phenylephrine was purchased from Elkins-Sinn Inc, Cherry Hill, NJ. L-NAME, L-arginine hydrochloride (L-Arg), D-arginine hydrochloride (D-Arg), acetylcholine, bradykinin, and sodium nitroprusside were purchased from Sigma Chemical Co, St Louis, Mo. Meclofenamate sodium was purchased from Calbiochem Corp, San Diego, Calif.

Experimental Protocol

Group 1 (n=6)

After a 10-day control period, L-NAME was infused intravenously at 7.4 nmol/kg per minute for 9 days. This was followed by a 7-day recovery period. The objective was to determine the long-term cardiovascular, hormonal, and renal excretory effects of inhibition of NO synthesis with L-NAME. Throughout the experiment arterial pressure, heart rate, water intake, urine volume, sodium intake, urinary sodium excretion, baroreceptor sensitivity, plasma renin activity, and the arterial pressure responses to intravenous injection of acetylcholine (5.5 nmol/kg), phenylephrine (12.2 μmol/kg), bradykinin (4.0 μmol/kg), and sodium nitroprusside (0.05 μmol/kg) were determined.

In preliminary experiments, dose-response relations were determined for acetylcholine, bradykinin, sodium nitroprusside, and phenylephrine. The doses chosen were near the middle of the upslope of this dose-response relation. With the use of these previously determined doses of sodium nitroprusside and phenylephrine, the heart rate component of baroreceptor reflex sensitivity was estimated by measuring the change in heart rate divided by the change in mean arterial pressure after infusion of either sodium nitroprusside or phenylephrine. These changes were determined when the change in heart rate was maximal.

Group 1a (n=14)

The objective was to determine whether the pressure and flow responses to acetylcholine, bradykinin, and sodium nitroprusside were changed after either acute meclofenamate administration or by a combination of a 6-day infusion of L-NAME plus acute meclofenamate administration. Data were collected with rats under sodium pentobarbital anesthesia.

Group 2 (n=4)

The objective was to determine whether L-Arg could reverse the sustained hypertension that remained after the L-NAME infusion was discontinued. After 5 days of control measurements, L-NAME was infused at 7.4 nmol/kg per minute for 9 days. Then L-Arg was infused intravenously at 9.5 μmol/kg per minute for 5 days. Mean arterial pressure, heart rate, and sodium and water balances were determined throughout the experiment.

Group 3 (n=6)

The objective was to measure the cardiovascular and renal responses to long-term L-Arg infusion. After 5 days of control measurements, a 7-day intravenous infusion of L-Arg (9.5 μmol/kg per minute) was started. Three days of recovery followed. Mean arterial pressure, heart rate, and sodium and water balances were determined during the control, L-Arg, and recovery periods.

Group 4 (n=7)

The objective was to determine whether L-Arg pretreatment could prevent L-NAME hypertension. After 5 days of control measurements, L-Arg was infused intravenously at 9.5 μmol/kg per minute for 4 days. On the second day of L-Arg, an intravenous L-NAME infusion (7.4 nmol/kg per minute) was begun and continued for 6 days. Mean arterial pressure, heart rate, and sodium and water balances were determined.

Group 5 (n=5)

The objective was to assess the specificity of L-NAME by determining whether D-Arg pretreatment could prevent L-NAME hypertension. After 5 days of control measurements, D-Arg was infused intravenously at 9.5 μmol/kg per minute for 4 days. On the second day of D-Arg, an intravenous L-NAME infusion (7.4 nmol/kg per minute) was begun and...
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Fig 1. Bar graphs show effects of continuous intravenous infusion of 7.4 nmol/kg per minute of N°-nitro-L-arginine methyl ester (L-NAME) on mean arterial pressure and heart rate of conscious rats. Arterial pressure and heart rate were continuously monitored through indwelling arterial catheters. *P<.05 compared with average control value.

Group 1: Changes in Mean Arterial Pressure and Heart Rate During L-NAME Infusion in Conscious Rats

Fig 1 shows that mean arterial pressure increased markedly during the infusion of 7.4 nmol/kg per minute L-NAME. On day 9 of L-NAME infusion, the mean arterial pressure was 150±7 mm Hg compared with an average control value of 121±3 mm Hg. During 7 days of recovery, the arterial pressure remained significantly elevated above its control value. Fig 1 also shows that heart rate had a tendency to decrease during L-NAME administration; however, these changes did not reach significance.

Group 1: Changes in Sodium and Volume Balances During L-NAME Infusion in Conscious Rats

Sodium intake was maintained at 2.8 mmol/d throughout the entire experiment. Fig 2 shows that there were no significant changes in sodium balance in either the L-NAME or recovery periods. Fig 2 also indicates that volume balance did not change significantly throughout the L-NAME and recovery periods. Therefore, NO synthesis inhibition with L-NAME in rats caused no net retention of either sodium or water.

Group 1: Changes in the Heart Rate Component of Baroreceptor Reflex Sensitivity During L-NAME Infusion in Conscious Rats

During the control period baroreceptor reflex sensitivity averaged 4.9±0.4 beats per minute (bpm) per millimeter of mercury during sodium nitroprusside administration and 1.9±0.4 bpm/mm Hg during phenylephrine administration. On day 6 of L-NAME (day L-6) the baroreceptor reflex sensitivity to sodium nitroprusside decreased to 3.7±0.5 bpm/mm Hg (P<.05). In addition, on day 5 of L-NAME (day L-5) the baroreceptor reflex sensitivity to phenylephrine significantly decreased to 1.3±0.4 bpm/mm Hg (P<.05).

Group 1: Arterial Pressure Responses to Vasoactive Drugs During L-NAME Infusion in Conscious Rats

Table 1 shows the pressure responses to acetylcholine, phenylephrine, bradykinin, and sodium nitroprusside during the control period, L-NAME, and recovery. None of these variables changed significantly during L-NAME or recovery. Therefore, the ability of the vasculature to dilate and constrict during L-NAME appears to be normal in these rats.
Group 1: Changes in Plasma Renin Activity During L-NAME Infusion in Conscious Rats

Fig 3 shows that the plasma renin activity of the conscious rats in this study averaged 1.9 ± 0.1 ng angiotensin I/mL per hour during the control period. On days 5, 7, and 9 of L-NAME, plasma renin activity was increased approximately 2.5-fold.

Group 1a: Cardiovascular Responses to Vasoactive Drugs During Meclofenamate or L-NAME Plus Meclofenamate Treatment

Table 2 shows the pressure and flow responses to acetylcholine, bradykinin, and sodium nitroprusside in conscious rats before and after acute meclofenamate treatment. Also, these responses are reported for rats after 6 days of continuous intravenous L-NAME infusion and in the same rats after acute meclofenamate treatment. There were no significant changes in the arterial pressure, iliac flow, or iliac resistance responses after any of the treatments.

Group 2: Reversal of L-NAME-Induced Mean Arterial Pressure and Heart Rate Changes and Sodium and Volume Balance Changes in Conscious Rats

The objective of the study in group 2 was to determine whether the sustained increase in arterial pressure during recovery from L-NAME could be reversed with L-Arg. Fig 4 shows that the arterial pressure increased significantly throughout the 9-day period of the continuous intravenous infusion of 7.4 nmol/kg per minute of L-NAME, and heart rate decreased. Intravenous L-Arg was infused continuously for the 5 days after L-NAME infusion at 9.5 μmol/kg per minute. The result, as seen in Fig 4, was a rapid and sustained reversal of the hypertension. Fig 4 also shows that the bradycardia that resulted during L-NAME was reversed during L-Arg infusion. In addition, sodium and volume balances did not change significantly during either the L-NAME or L-Arg periods.

Group 3: Changes in Mean Arterial Pressure, Heart Rate, Sodium Balance, and Volume Balance During L-Arginine Infusion in Conscious Rats

The objective of the study in group 3 was to determine the cardiovascular and renal excretory responses to a continuous intravenous L-Arg infusion equivalent to the rate used in group 2. As seen in Fig 5, L-Arg caused no significant changes in either mean arterial pressure or heart rate. In addition, Fig 6 shows that neither sodium balance nor volume balance was significantly changed by the L-Arg infusion.

Group 4: Changes in Mean Arterial Pressure, Heart Rate, and Sodium and Volume Balances During L-NAME Infusion in Conscious Rats Pretreated With L-Arginine

The objective of the study in this group of rats was to determine whether L-Arg pretreatment could prevent L-NAME hypertension. L-Arg and L-NAME were infused at the same rates as in groups 1 through 3. As seen in Fig 7, L-Arg alone caused no changes in either mean arterial pressure or heart rate on day 1. When L-Arg and L-NAME were infused together on days 2 through 4, no change in either arterial pressure or heart rate occurred. On the other hand, when the L-Arg infusion was stopped on day 5 and L-NAME was infused alone, mean arterial pressure increased and heart rate decreased similar to the responses in groups 1 and 2. In addition, sodium and volume balances did not change significantly during administration of either L-Arg or L-NAME alone or in combination.

Group 5: Changes in Mean Arterial Pressure, Heart Rate, Sodium Balance, and Volume Balance in Conscious Rats Pretreated With D-Arginine

Group 4 showed that intravenous L-Arg infusion prevented L-NAME–induced hypertension; therefore, to test the specificity of L-NAME, we repeated the experiments and substituted D-Arg for L-Arg. D-Arg was infused at 9.5 μmol/kg per minute on days 1 through 4. As shown in Fig 8, on day 1 no changes in either mean arterial pressure or heart rate occurred during D-Arg infusion, but when D-Arg was infused in combination with L-NAME at 7.4 nmol/kg per minute, arterial pressure increased significantly and heart rate decreased significantly. Infusion of L-NAME alone on days 5 through 7 resulted in hypertension, as in the previous groups. Also, neither sodium nor volume balance was significantly changed during administration of either D-Arg, L-NAME, or a combination of D-Arg and L-NAME. Therefore, L-Arg prevented L-NAME hypertension, but the same molar quantities of D-Arg did not.
TABLE 2. Cardiovascular Responses to Acetylcholine, Bradykinin, and Sodium Nitroprusside In Anesthetized Rats

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Stimulus</th>
<th>Control</th>
<th>Meclo</th>
<th>L-NAME</th>
<th>L-NAME+Meclo</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP responses, % change</td>
<td>ACh, IV</td>
<td>-42±2</td>
<td>-44±3</td>
<td>-35±2</td>
<td>-36±6</td>
</tr>
<tr>
<td></td>
<td>BK, IV</td>
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<td>-25±6</td>
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<tr>
<td></td>
<td>SNP, IV</td>
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<td>-50±3</td>
<td>-59±8</td>
<td>-52±8</td>
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<tr>
<td>Integ flow, µL</td>
<td>ACh, IA</td>
<td>264±55</td>
<td>312±126</td>
<td>229±67</td>
<td>306±131</td>
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<tr>
<td></td>
<td>BK, IA</td>
<td>771±162</td>
<td>658±329</td>
<td>639±104</td>
<td>528±230</td>
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<tr>
<td></td>
<td>SNP, IA</td>
<td>555±165</td>
<td>729±307</td>
<td>510±172</td>
<td>651±260</td>
</tr>
<tr>
<td>Maximum iliac flow, % change</td>
<td>ACh, IA</td>
<td>21±10</td>
<td>45±11</td>
<td>37±5</td>
<td>29±8</td>
</tr>
<tr>
<td></td>
<td>BK, IA</td>
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<td>47±19</td>
<td>32±4</td>
<td>30±7</td>
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<tr>
<td></td>
<td>SNP, IA</td>
<td>24±3</td>
<td>34±13</td>
<td>37±12</td>
<td>32±13</td>
</tr>
<tr>
<td>Iliac resistance, % change</td>
<td>ACh, IA</td>
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<td>-35±6</td>
<td>-32±3</td>
<td>-27±7</td>
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<tr>
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<td>-34±7</td>
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</tr>
<tr>
<td></td>
<td>SNP, IA</td>
<td>-25±2</td>
<td>-36±5</td>
<td>-32±9</td>
<td>-26±8</td>
</tr>
</tbody>
</table>

Meclo indicates meclofenamate sodium; L-NAME, Nω-nitro-L-arginine methyl ester; MAP, mean arterial pressure; Integ flow, area under the iliac artery flow-time curve; ACh, acetylcholine; BK, bradykinin; and SNP, sodium nitroprusside. Data are mean±SEM. Intravenous dosages were ACh, 5.5 nmol/kg; BK, 4.0 nmol/kg, and SNP, 0.05 µmol/kg. Intravenous dosages were ACh, 0.55 nmol/kg; BK, 0.40 nmol/kg; and SNP, 0.05 nmol/kg. Control values were obtained 20 minutes after meclo administration of 15.7 µmol/kg IV to the control group. L-NAME values were obtained in six rats after 7.4 nmol/kg per minute IV L-NAME for 6 days. L-NAME and meclo values were determined 20 minutes after meclo injection of 15.7 µmol/kg IV. There were no significant differences in any of the groups.

Group 6: Changes in Mean Arterial Pressure and Heart Rate When a Higher Dose of L-NAME Was Administered to Conscious Rats

To determine whether NO synthesis was maximally inhibited during the infusion of 7.4 nmol/kg per minute

L-NAME, we infused a dose of 37 nmol/kg per minute IV for 9 days in another group of rats. As seen in Fig 9, arterial pressure rapidly increased to hypertensive levels, and on day 9 of L-NAME the arterial pressure had increased 31 mm Hg over its control pressure. The daily percent increases in arterial pressure of this group were compared with the percent pressure increases of group 1, which received the lower dosage of L-NAME (Fig 1). The 37 nmol/kg per minute dose of L-NAME caused a
faster increase in arterial pressure on days 1 and 2 of L-NAME ($P<.05$), but after day 2 the percent increases in pressure of the two groups were not different. Therefore, if the increase in arterial pressure during L-NAME administration is used as an index of NO synthesis inhibition, the lower dose of 7.4 nmol/kg per minute L-NAME may have caused full inhibition of NO synthesis.

Discussion

Responses of Arterial Pressure to Nitric Oxide Synthesis Inhibition

NO synthesis was chronically inhibited in Sprague-Dawley rats by continuous intravenous infusion of L-NAME at 7.4 nmol/kg per minute for 9 days, and at this time arterial pressure had increased by 29 mm Hg.

When the L-NAME infusion rate was increased fivefold in another group of rats, arterial pressure increased more rapidly, but the final increase in arterial pressure was the same as in the group with the lower infusion rate. Thus, this is the first demonstration, to our knowledge, that long-term intravenous infusion of NO synthesis inhibitors causes chronic hypertension in normal rats.

Previous studies by other investigators have shown that oral administration of NO synthesis inhibitors causes hypertension in rats. Gardiner et al. first showed that oral administration of either $N^\omega$-monomethyl L-arginine (L-NMMA) or L-NAME caused increased arterial pressure for 9 hours in Brattleboro rats. Subsequent studies by the same group showed that hypertension...
induced by oral L-NMMA could persist for up to 7 days in Brattleboro rats, and oral L-NAME could cause increased arterial pressure lasting up to 6 months. Baylis et al. confirmed those studies by showing that oral L-NMMA treatment of rats at 18.5 μmol/kg per day for 2 months caused hypertension. Using a higher dosage of L-NAME of 222 μmol/kg per day for 4 to 6 weeks, Ribeiro et al. found that arterial pressure, measured by the tail-cuff technique, was increased. The present study showed that a chronic intravenous infusion of L-NAME could cause chronic hypertension in normal rats. The arterial pressures in this study were measured continuously for 21 h/day in indwelling catheters, which should give a very accurate estimate of arterial pressure.

Administration of L-NAME to conscious dogs also has been shown to cause hypertension. Persson et al. found that a single intravenous injection of 61 μmol/kg L-NAME caused arterial pressure to increase for up to 24 hours. Manning et al. found that a continuous intravenous infusion of 37.1 nmol/kg per minute of L-NAME for 11 days into conscious dogs caused chronic hypertension that could be reversed partially with L-Arg. Therefore, we have found that continuous intravenous infusion of L-NAME causes chronic hypertension in both normal rats (present study) and dogs using constant measurement of arterial pressure.

Whether this hypertension is due to decreases in NO synthesis depends on the specificity of the inhibitor. Two basic approaches have been used in previous studies to test this specificity. First, the ability of the NO precursor L-Arg to prevent or reverse this hypertension is tested. This is usually compared with the D-Arg response. Second, the ability to block the depressor or hyperemic responses to an NO agonist such as acetylcholine is tested. In the present study, L-Arg was infused intravenously along with L-NAME, and this addition of L-Arg prevented the normal L-NAME-induced increase in arterial pressure. When D-Arg was substituted for L-Arg in the same protocol, arterial pressure increased during the combined infusion of D-Arg and L-NAME. This demonstrated the stereospecificity of L-NAME for inhibiting NO production from L-Arg. In time-control studies in another group of rats, this same dose of L-Arg was infused intravenously for 7 days, and no change in arterial pressure occurred. In addition, the present study also showed that L-Arg quickly reversed the sustained increase in arterial pressure after L-NAME was discontinued. Other investigators have also shown that L-Arg partially reverses L-NAME hypertension in both dogs and rats. Therefore, several studies suggest that the increases in arterial pressure after long-term L-NAME administration are due to inhibition of the L-Arg-NO pathway.

The specificity of L-NAME to inhibit NO synthesis also has been tested in several studies by infusion of NO agonists. Most studies have found that it is difficult to attenuate either the acetylcholine or bradykinin depressor or vasodilator response during NO inhibition in intact animals. Tresham et al. found that a bolus injection of L-NAME of 37 μmol/kg caused hypertension but did not alter the acetylcholine depressor response. Aisaka et al. found that an intravenous bolus administration of 20.6 μmol/kg L-NMMA did not alter the magnitude of the acetylcholine depressor response, but the duration of the response decreased. O'Shaughnessy et al. found that the hypotensive actions of either acetylcholine or bradykinin were unaffected by L-NAME administration in anesthetized rats. Zambetis et al. found only a marginal inhibitory effect of the acetylcholine depressor response in anesthetized rats during L-NAME administration. This response was unaffected by glibenclamide, suggesting that hyperpolarization of vascular smooth muscle via activation of the ATP-sensitive potassium channels is not an important mediator of the acetylcholine depressor response. On the other hand, some investigators have found inhibition of the acetylcholine hypotensive effects during short-term L-NAME administration in anesthetized rats and during long-term L-NAME administration in conscious dogs. Gardiner et al. found attenuation of the bradykinin hypotensive effects, but not the acetylcholine effects, during short-term L-NAME administration. The bradykinin effects on vasodilation of the renal and hindquarter beds, but not the acetylcholine effects, were attenuated during L-NAME administration. When neurohumoral plus baroreceptor reflex responses were blocked in these rats, the vasodilator and vasodepressor effects of acetylcholine were significantly attenuated by L-NAME.

We also have recently shown that blockade of the baroreceptor reflex and neurohumoral systems in conscious rats that had been intravenously infused with 7.4 nmol/kg per minute L-NAME for 9 days caused significant attenuation of the acetylcholine depressor effect. In the present study, we failed to find a decrease in either the acetylcholine or bradykinin depressor or vasodilator effects during L-NAME administration. The data from our study and the study of Gardiner et al. suggest that the effects of acetylcholine on arterial pressure have either a non-endothelium-dependent component or that cardiovascular reflexes severely interfere with the response.

A recent study in conscious primates showed that the increases in arterial pressure with either a 37.1 μmol/kg or 371 μmol/kg IV bolus of L-NAME were similar. However, the acetylcholine depressor effect was inhibited only at the higher dose of L-NAME, but this higher dose could have caused muscarinic blockade. The authors concluded that the basal release of NO from vascular endothelium can be inhibited at doses lower than those required to inhibit its release stimulated by acetylcholine.

Even though the in vivo effects of NO agonists on vascular resistance and arterial pressure have shown little attenuation during NO synthesis inhibition in most studies on intact rats, the in vitro studies have shown quite different results. In a recent study by Kobayashi et al., L-NAME was given in the food of rats for 1 week in the same daily amount as the amount infused in the present study, and systolic pressure measured by the tail-cuff method increased 25 mm Hg. This degree of hypertension is similar to that seen in the present study. The acetylcholine effects on vascular relaxation of aortic rings studied in organ baths were significantly attenuated in Kobayashi's L-NAME-treated rats. Therefore, it is likely that NO synthesis was decreased in their study as well as in the present study because the amount of L-NAME administered was the same in the two studies and the level of hypertension was similar.
Mechanism of Hypertension During Nitric Oxide Synthesis Inhibition

A number of experiments have demonstrated that long-term increases in arterial pressure are associated with changes in either sodium and water intake or renal function. The sodium intake was fixed in the present study, and neither sodium nor volume balance was changed during L-NAME administration. Thus, the long-term renal pressure-natriuresis relation may have shifted to the right along the arterial pressure axis during L-NAME infusion.

Lahera et al23 have provided evidence that L-NAME shifts the pressure-natriuresis relation. They recently infused L-NAME intravenously into anesthetized rats in doses ranging from 0.37 to 185 nmol/kg per minute, and the administration of 3.7 nmol/kg per minute L-NAME decreased both sodium excretion and renal plasma flow without a change in arterial pressure. However, when the L-NAME infusion rate was increased to 37 nmol/kg per minute, mean arterial pressure increased, which overcame the tendency of L-NAME to cause sodium retention. These data suggest that the lack of change in sodium balance in the present experiment during L-NAME administration could have been caused by the opposing effects of NO synthesis inhibition and arterial pressure on urinary sodium excretion.

Several other investigators have shown that NO synthesis inhibition results in renal vasoconstriction, which could be responsible for a putative shift in the pressure-natriuresis relation. During oral L-NAME administration for 9 hours in rats, renal vascular resistance increased significantly. In longer studies, oral administration of L-NAME to rats for either 4 to 6 weeks or 2 months caused decreases in glomerular filtration rate and renal plasma flow and an increase in renal vascular resistance. In studies in dogs, a very low dose of L-NAME of 0.185 nmol/kg per minute caused no change in arterial pressure but a decrease in glomerular filtration rate and renal plasma flow without a change in arterial pressure. However, when the L-NAME infusion rate was increased to 37 nmol/kg per minute, mean arterial pressure increased, which overcame the tendency of L-NAME to cause sodium retention. These data suggest that the lack of change in sodium balance in the present experiment during L-NAME administration could have been caused by the opposing effects of NO synthesis inhibition and arterial pressure on urinary sodium excretion.

During L-NAME infusion we found no overall change in sodium and water balances, and this has been shown previously in dogs. Thus, we suggest that fluid volumes were unchanged during NO synthesis inhibition. Therefore, L-NAME-induced hypertension may be a vasoconstrictor type of hypertension and not a volume-dependent hypertension. Indeed, other studies have shown that L-NAME administration results in increased vascular resistance in the renal, hindquarter, and mesenteric circulation in the rat. In fact, L-NAME infusion has been shown to cause decreases in cardiac output and increases in total peripheral resistance in both rats and dogs. Therefore, a number of studies support the theory that NO synthesis inhibition causes a vasoconstrictor type of hypertension.

Even though it is reasonably clear that L-NAME administration causes long-term vasoconstriction and hypertension, the exact mechanisms involved in these responses are not clear. Because NO causes vasodilation through the cyclic GMP (cGMP) pathway, blockade of NO production could lead to a decrease in cGMP and thus vasoconstriction and an increase in arterial pressure. In addition, NO has been shown to interact at least acutely with several other vasoconstrictor mechanisms, and these mechanisms could mediate part of the vasoconstrictor/hypertensive response.

One possible mediator of the L-NAME-induced hypertension is an increase in angiotensin II concentration. Salazar et al26 demonstrated that plasma renin activity increased during very low doses of L-NAME in conscious dogs, but studies in our laboratory in dogs found that long-term L-NAME administration of 37.1 nmol/kg per minute for 6 days caused no increase in plasma renin activity. Ribeiro et al27 found that renin activity increased in rats receiving high doses of L-NAME orally for 4 weeks and that angiotensin II antagonism with losartan attenuated the increase in arterial pressure. On the other hand, Samsell et al28 found that acute administration of losartan to rats on chronic L-NAME did not cause a decrease in arterial pressure. In the present study, intravenous administration of L-NAME resulted in a 2.5-fold increase in plasma renin activity, which could have mediated part of the increase in arterial pressure.

Another possible mediator of the increase in arterial pressure during L-NAME administration is an increase in sympathetic activity. Sakuma et al31 recently showed that renal sympathetic artery activity acutely increased after administration of L-NAME. However, Samsell et al28 found that arterial pressure increased 36% in rats on chronic L-NAME and 37% in rats on L-NAME plus prazosin. In addition, Pucci et al32 found that the pressor and renal vasoconstrictor effects of L-NAME were not impaired in anesthetized rats with blockade of either ganglionic transmission, alpha-adrenergic receptors, arginine vasopressin, the renin-angiotensin system, or prostanooids. Therefore, whether increased sympathetic activity mediates part of the L-NAME pressor effect in the present study is not clear.

Another possible mediator of the L-NAME pressor effects could be enhanced vasoconstrictor sensitivity of vascular smooth muscle to vasoconstrictors or a decreased sensitivity to vasodilators. As seen in Table 1, the phenylephrine sensitivity was unchanged during L-NAME in this experiment, which is evidence against any enhanced vasoconstrictor sensitivity. A previous study in rats33 showed that norepinephrine sensitivity increased acutely after NO synthesis inhibition with L-NMMA. However, in dogs on chronic L-NAME for 11 days, we showed that phenylephrine sensitivity was increased only on the second day of L-NAME administration. The depressor sensitivity of bradykinin and sodium nitroprusside were unchanged in the present experiment, which is evidence against any deficit in vasodilator ability contributing to an L-NAME-induced increase in arterial pressure.

An increase in endothelium-derived contracting factors such as thromboxane A2 or prostaglandin H2 during L-NAME could have contributed to an increase in arterial pressure. However, the arterial pressure and renal hemodynamics during acute L-NAME have recently been shown to be independent of changes in
prostanoids. Whether prostanoids contribute to long-term changes in arterial pressure in the present experiment was not directly addressed by our protocols.

Finally, changes in baroreceptor reflex gain could have mediated part of the increase in arterial pressure in this experiment. However, it was recently shown that baroreceptor reflex gain during nitroprusside administration was decreased only moderately during an 11-day L-NAME infusion into dogs. In the present experiment, the baroreceptor reflex sensitivity was also moderately decreased between days 5 and 6 of L-NAME. This could have had a transient effect on arterial pressure, but this decrease in sensitivity was not sustained.

Although evidence suggests that hypertension during NO synthesis inhibition involves systemic vasoconstriction, the mechanism of hypertension remains unclear. However, despite the absence of sodium or volume retention, it is important to note that a decrease in renal excretory capability also accompanied chronic NO synthesis inhibition. This is evidenced by the fact that the rats were in sodium balance at an elevated arterial pressure, indicating that pressure natriuresis was shifted to a higher pressure. Without this shift, systemic vasoconstriction could not have chronically elevated arterial pressure because sodium and water excretions would have increased until blood pressure returned to control. One potential mechanism for this shift is an increase in renal vascular resistance.

In group 1, arterial pressure remained elevated 1 week after the L-NAME infusion was discontinued. Baylis et al demonstrated that arterial pressure returned to control within 48 hours after a 6-month oral L-NAME administration was stopped. However, Ribeiro et al showed that arterial pressure remained elevated for more than 2 weeks after a 4- to 6-week oral L-NAME treatment was stopped. Long-term L-NAME treatment may result in prolonged NO synthesis inhibition, but our data showed that L-Arg administration can quickly reverse this inhibition.

In summary, NO synthesis inhibition by continuous intravenous infusion of L-NAME at 7.4 nmol/kg per minute for 9 days in conscious rats caused a sustained increase in mean arterial pressure. A fivefold increase in the L-NAME infusion rate for 9 days in another group of rats caused no greater increase in arterial pressure. During L-NAME administration at 7.4 nmol/kg per minute, plasma renin activity increased 2.5-fold, sodium and water balances were unchanged, the pressor sensitivity to phenylephrine was unchanged, and the vasodilpressor and vasodilator responses to acetylcholine and bradykinin were unchanged. Continuous intravenous L-Arg administration, at a rate that had no effect on arterial pressure or sodium balance, quickly reversed the sustained increase in arterial pressure that occurred after L-NAME administration was stopped. In addition, L-Arg when infused in conjunction with L-NAME prevented any increase in arterial pressure, but D-Arg did not prevent the L-NAME-induced hypertension.

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