Cardiovascular Responses to Long-term Blockade of Nitric Oxide Synthesis

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The goal of this study was to determine if there is a basal release of nitric oxide that affects long-term arterial pressure regulation in dogs. Studies were conducted over a 23-day period in eight conscious dogs with indwelling catheters. Nitric oxide synthesis was blocked by continuous intravenous infusion of nitro-L-arginine-methyl ester at 37.1 nmol/kg per minute for 11 days. Arterial pressure increased to 120±4% of control on the first day, decreased for a few days, and then increased to a maximum value of 122±6% of control on day 7. Bradycardia was sustained throughout the entire nitro-arginine period. Blockade of nitric oxide synthesis was evidenced by attenuated pressure and flow responses to systemic acetylcholine infusion. The pressor response to phenylephrine was increased for only 1 day, and the hypotensive effects of nitroprusside were enhanced. Also, the variability of arterial pressure was significantly increased during nitro-arginine. Sodium and water balances were positive the first day of nitro-arginine infusion but were unchanged for the entire nitro-arginine period. In conclusion, the data suggest that blockade of the basal release of nitric oxide in dogs causes an increase in the long-term level of arterial pressure without any sustained sodium or water retention. (Hypertension 1993;22:40-48)

KEY WORDS • endothelium • vasodilation • arginine • nitric oxide • hypertension, essential

In 1980 Furchgott and Zawadzki found that acetylcholine-induced relaxation of vascular strips or rings was dependent on the presence of an intact endothelium. A substance released from the endothelium, later known as endothelium-derived relaxing factor (EDRF), was primarily responsible for this relaxation. Furchgott later suggested that EDRF was nitric oxide (NO), and Ignarro et al speculated that EDRF might be NO or a related compound. Other investigators have confirmed that NO is an integral part of EDRF, and specific NO synthesis inhibitors have been successfully developed.

The two major inhibitors of NO production are N\textsubscript{\textsuperscript{\text{\textdegree}}}monomethyl L-arginine (L-NMMA) and N\textsubscript{\textsuperscript{\text{\textdegree}}}nitro-L-arginine-methyl ester (L-NAME), and they both act by competitive inhibition of NO synthase. This inhibition prevents the conversion of L-arginine to NO. Of these two inhibitors, L-NAME is much more potent and causes acute vasoconstriction in a number of vascular beds, whereas D-NAME does not.

Most experiments on NO synthesis blockade have been performed in vitro on vascular rings. However, more recently, administration of NO synthesis blockers resulted in acute increases in arterial pressure in rats, guinea pigs, and rabbits. In addition, studies by Gardner et al and Baylis et al showed that administration of L-NAME for several hours to conscious rats resulted in increases in arterial pressure. More recently, several longer experiments have been performed in which NO synthesis inhibitors were administered to rats orally for periods lasting between 9 hours and 6 months, and mean arterial pressure increased markedly in each study. This NO inhibition in rats was associated with a decrease in cardiac output and blood flow to several vascular beds, and the bradycardia that accompanied the NO inhibition was reversed with atropine. These studies suggest strongly that NO synthesis inhibition in the rat has marked effects on arterial pressure regulation for both short-term (minutes and hours) and long-term (days and weeks) periods. NO synthesis inhibitors have also been administered to conscious dogs by Persson et al, who found that a single injection of L-NAME caused an increase in arterial pressure that lasted for 24 hours, and by Salazar et al, who found that a very low dose of L-NAME for 3 days caused renal vasoconstriction but no increase in mean arterial pressure. However, whether long-term systemic NO synthesis inhibition will result in long-term increases in arterial pressure in dogs is not known. Therefore, our goal was to determine the cardiovascular effects of NO synthesis inhibition in dogs for 11 days. Additional goals were to determine if long-term NO synthesis inhibition results in changes in the pressure responses to vasoactive agents, sodium and water balance, and the variability of arterial pressure and heart rate. Also, the responses of arterial pressure and the pressure sensitivity of vasoactive agents to L-arginine were determined.

Methods

Animal Preparation and Experimental Protocol

Experiments were performed on eight conscious dogs with an average body weight of 24.3±0.6 kg. The project was approved by the local Institutional Animal Committee. Aseptic technique was used on surgical procedures,
Nitric Oxide and Cardiovascular Function

**Short-term Effects of L-Arginine on Cardiovascular Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>L-NAME</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>P</td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>79.1±2.2</td>
<td>81.0±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>PE pressor response (mm Hg)</td>
<td>21.0±4.8</td>
<td>16.9±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>SNP depressor response (mm Hg)</td>
<td>-20.9±2.1</td>
<td>-20.1±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Baroreceptor sensitivity to SNP (bpm/mm Hg)</td>
<td>4.2±0.9</td>
<td>3.0±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Baroreceptor sensitivity to PE (bpm/mm Hg)</td>
<td>1.4±0.5</td>
<td>1.5±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>ACh flow response (mL)</td>
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L-NAME, nitro-L-arginine-methyl ester; PE, phenylephrine; SNP, sodium nitroprusside; bpm, beats per minute; ACh, acetylcholine.

Data are reported as mean ± SEM. Differences between means were compared using the Student’s t-test. Data are reported in the Table. The effects of L-arginine on the acetylcholine flow response were not determined during the control period because of technical problems in recording blood flow. L-Arginine was also infused acutely on days 9 through 11 of L-NAME, and the effects on arterial pressure were determined.

Experimental Methods and Instrumentation

The dogs were housed in metabolic cages and were fitted with a backpack that held a Statham P23 AC or P23 ID transducer at the level of the heart. The aortic and atriopeptide (1 mL of 0.58 μmol/mL IM; Elkins-Sinn, Cherry Hills, NJ) and acepromazine maleate (22.6 μmol IM; Tech America, Elwood, Kan) were administered before surgery. Anesthesia was initiated with thiopental sodium (Pentothal, 94.6 μmol/kg IV; Abbott, North Chicago, Ill) and maintained with a mixture of methoxyflurane (Penthrane, Abbott) and oxygen. Appropriate gas concentrations were delivered to the dogs from an anesthesia machine (Kinnet-o-meter, Ohio Medical Products, Madison, Wis) through an endotracheal tube. Stadol (4.2 μmol IM; Bristol Laboratories, Evansville, Ind) was administered for analgesia after surgery as needed.

During surgery catheters were implanted in the aorta and inferior vena cava through the femoral artery and vein. The catheters were tunneled subcutaneously and exited in the back between the dogs’ shoulders. In four dogs, electromagnetic flow probes (Zepeda, Seattle, Wash) and a pneumatic occluder were placed around the external iliac artery via a flank incision. The flow probe wires and the occluder tube exited the back caudal to the shoulders. A Zepeda flowmeter was used to measure the response of iliac arterial blood flow to intra-arterial acetylcholine. A recovery period of 3 weeks was allowed for the flow probes to stabilize, and this time was used to train the dogs to lie quietly in their cages.

Water intake was ad libitum throughout the experiment. Sodium intake was maintained at 35 to 45 mmol/d during the entire experiment by feeding the dogs 894 g/d of K-D Prescription Diet dog food (Hill’s Pet Products, Topeka, Kan), which provided 30 mmol of sodium and 26.8 mmol of potassium per day; the sodium intake from any infusions was added to the dietary intake to determine the total sodium intake. Volume balance was calculated by adding the water intake by drinking and the volume of solutions infused and subtracting urinary volume excretion. However, there is some water content in the food that adds to volume balance and some fecal and insensitive loss of water that 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, giving a more accurate estimate of the true balance. Sodium balances were calculated in a similar way.

After the surgical recovery period, measurements of arterial pressure, the short-term pressure responses to vasoactive drugs, baroreceptor reflex sensitivity, plasma protein concentration, plasma electrolyte concentrations, and the iliac flow responses to intra-arterial acetylcholine infusion were determined over a 7-day control period. Then, an intravenous infusion of L-NAME (37.1 nmol/kg per minute, Sigma Chemical Co, St Louis, Mo) was begun and continued for 11 days. This was immediately followed by a 6-day recovery period. Throughout the experiment, acetylcholine (1.38 μmol in five dogs or 2.06 μmol in three dogs, Sigma) was injected intravenously or intra-arterially in a bolus form. The intravenous acetylcholine was used to elicit a depressor response, whereas the intra-arterial acetylcholine went directly into the distal aorta and elicited a local increase in blood flow in the hind leg. Phenylephrine HCl (0.49 μmol IV bolus, Elkins-Sinn) and sodium nitroprusside (3.26 μmol IV bolus, Sigma) were also acutely injected intravenously, and the pressure responses were determined. These depressor or pressor responses are reported in Fig 3 and were determined before any acute infusions of L-arginine were given.

On control day −3, day 8 of L-NAME infusion, and day 15 during the recovery period, L-arginine hydrochloride (1.42 mmol/kg IV, Sigma) was infused once each day in a 30-minute period, and the responses of arterial pressure, the pressor response to phenylephrine injection (0.49 μmol IV), the depressor response to nitroprusside injection (3.26 μmol IV), the baroreceptor reflex sensitivities to phenylephrine and nitroprusside, and the area under the iliac flow-time curve after intra-arterial acetylcholine were determined both before the L-arginine infusion and 15 to 45 minutes after its infusion. These data are reported in the Table. The effects of L-arginine on the acetylcholine flow response were not determined during the control period because of technical problems in recording blood flow. L-Arginine was also infused acutely on days 9 through 11 of L-NAME, and the effects on arterial pressure were determined.
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24-HOUR MAP (% control)

24-HOUR HEART RATE (% control)

FIG 1. Bar graphs show effects of intravenous infusion of 37.1 nmol/kg per minute of nitro-L-arginine-methyl ester (L-NAME) on 24-hour average of mean arterial pressure (MAP) and heart rate. On days –3, 8 through 11, and 15, 1.42 mmol/kg of L-arginine hydrochloride (L arg) was infused intravenously in 30 minutes, causing arterial pressure to decrease on days 8 through 11 from its maximum value on day 7. *P<.05 compared with average control value.

c = 561 ml

The L-NAME infusion caused two dogs to become constipated, so on day 7 of the L-NAME infusion, the catheter was connected to the pressure transducer, thus allowing continuous pressure recordings. The transducer wires were connected to a model 7D recorder (Grass Instrument Co, Quincy, Mass) that was connected to a digital computer. Data were sampled at 200 Hz for 12 seconds every minute throughout the day, and arterial pressure and heart rate were determined each minute and stored on a computer disk. Also, customized software enabled us to sample arterial pressure and iliac flow rapidly during the intra-arterial infusion of acetylcholine. These data were analyzed, and the area under the iliac arterial flow-time curve above baseline after intra-arterial acetylcholine injection was determined. The calculation of this area began when iliac flow increased above baseline and continued until flow returned to control (approximately 20 to 30 seconds). This provided the total volume of blood that flowed through the iliac artery due to the infusion of acetylcholine.

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 injection of either sodium nitroprusside or phenylephrine, and these changes were determined when the heart rate change was maximal. The values of baroreceptor reflex sensitivity reported in Fig 4 were determined before any acute administration of L-arginine. Daily standard deviations (SD) of arterial pressure and heart rate were calculated from the data collected each minute by the computerized monitoring system. Plasma and urine sodium and potassium concentrations were determined by flame photometry, and plasma protein concentration was determined with a refractometer (American Optical, Buffalo, NY).

The L-NAME infusion caused two dogs to become constipated, so on day 7 of the L-NAME infusion through the end of the experiment, data from one dog was eliminated. Data from the other dog was eliminated from day 8 through the end of the experiment. Four dogs received a small amount of psyllium hydrophilic muciloid (Metamucil, 2.5 g; Procter & Gamble, Cincinnati, Ohio) with their food twice a week throughout the experiment, and this prevented any constipation.

Statistical analysis was performed by first determining overall significance with analysis of variance for repeated measures. Significance on individual experimental days was determined post hoc with Dunnett’s Test for multiple comparisons with a control. All dogs served as their own controls, and experimental and recovery data were statistically compared with the average data from the entire control period. A paired t test was used to compare the acute effects of L-arginine on several cardiovascular variables. Data were considered to be statistically different from control at a value of P<.05.

Results
Changes in Mean Arterial Pressure and Heart Rate During L-NAME Infusion

Fig 1 shows that mean arterial pressure increased significantly on the first day of L-NAME infusion. The control value of mean arterial pressure was 78.5±3.1 mm Hg. On day 1 the arterial pressure had increased to 120±4% of control (P<.05) and remained significantly elevated throughout the remainder of the L-NAME period except on day 9.

To determine if L-arginine could reverse the L-NAME effect on arterial pressure, on day –3 of the control period, days 8 through 11 of the L-NAME period, and day 15 of the recovery period, we infused 1.42 mmol/kg IV of L-arginine each day in a 30-minute period. No significant change in the daily average arterial pressure occurred on days –3 or 15. However, arterial pressure significantly decreased on each of days 8 through 11 when compared with day 7.

On days 12 and 13 during the recovery period, arterial pressure remained significantly elevated; however, after day 13 the pressure was not significantly
Fig 2. Bar graphs show effects of intravenous infusion of nitro-L-arginine-methyl ester (L-NAME) on change in iliac flow responses and area under the iliac flow-time curve after bolus intra-arterial injection of acetylcholine (ACH) into conscious dogs. Attenuated flow responses suggest effective nitric oxide synthesis blockade. On days −3, 8 through 11, and 15, 1.42 mmol/kg of L-arginine was infused intravenously in 30 minutes. However, responses indicated in this figure were measured before L-arginine infusion. *P<.05 compared with average control value.

Responses of Iliac Arterial Flow and the Area Under the Iliac Flow-Time Curve After Acetylcholine Infusion

Fig 2 demonstrates that during the control period the iliac arterial blood flow increased an average of 658±329 mL/min after intra-arterial acetylcholine infusion. Control resting iliac flow was 69±14 mL/min. The iliac flow response to acetylcholine was significantly decreased during the entire L-NAME period.

Also shown in Fig 2 are the changes in the area under the iliac arterial blood flow-time curve after intra-arterial acetylcholine administration. This variable should be a good index of the total NO release due to acetylcholine infusion, even though part of the acetylcholine effect could have been due to vasodilator prostaglandins or other factors. The average area during the control period was 194±97 mL, and this represents the total extra volume of blood that flowed through the iliac artery after intra-arterial acetylcholine infusion. The area was significantly decreased on every day during the L-NAME infusion period, which suggests that NO release in response to acetylcholine was decreased well below its control value.

Changes in the Arterial Pressure Responses to Acetylcholine, Sodium Nitroprusside, and Phenylephrine During L-NAME Infusion

Fig 3 shows that the decrease in arterial pressure after intravenous acetylcholine infusion averaged −23±3 mm Hg during the control period. By day 3 the arterial pressure change was significantly attenuated and had reached a value of −6±3 mm Hg (P<.05). The arterial pressure response to acetylcholine remained significantly depressed throughout L-NAME.

Fig 3 also shows the responses of arterial pressure to nitroprusside. The mean pressure change during the control period was −18±2 mm Hg, and the depressor responses to nitroprusside during the L-NAME period were significantly greater after day 3. Fig 3 also shows the pressor responses to phenylephrine during the experiment. The mean control pressure change was 21±3 mm Hg. On day 2 the arterial pressure change was 34±4 mm Hg (P<.05), but the changes in pressure during the remainder of L-NAME were not significantly different from control.

Changes in the Heart Rate Component of Baroreceptor Reflex Sensitivity During L-NAME Infusion

Changes in baroreceptor reflex sensitivity during nitroprusside and phenylephrine administration are
8.0
6.5
5.0
3.5
2.0
0.0
BAROREFLEX SENSITIVITY TO
NITROPRUSSIDE (beats/min/mmHg)

6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
BAROREFLEX SENSITIVITY TO
PHENYLEPHRINE (beats/min/mmHg)

FIG 4. Bar graphs show effects of intravenous infusion of nitro-L-arginine-methyl ester (L-NAME) on heart rate component of baroreceptor reflex sensitivity during administration of either nitroprusside or phenylephrine in conscious dogs. On days —3, 8 through 11, and 15, 1.42 mmol/kg of L-arginine was infused intravenously in 30 minutes. However, responses indicated in this figure were measured before L-arginine infusion. *P<.05 compared with average control value.

Changes in Sodium Intake, Urinary Sodium Output, and Sodium Balance During L-NAME Infusion

Fig 6 shows that the mean sodium intake during the control period was 36±3 mmol/d. Sodium intake was maintained very close to this control intake throughout the remainder of the experiment. Urinary sodium output, also shown in Fig 6, had a mean control value of 27±3 mmol/d. On day 1 of L-NAME infusion, urinary sodium excretion decreased markedly to 6±2 mmol/d (P<.05). However, by day 2 the sodium output had returned to control, and for the entire period of L-NAME infusion there were no significant changes in urinary sodium output. Fig 6 also shows the changes in sodium balance during L-NAME infusion. Sodium balance increased from a control value of 0.0±3 to 20±2 mmol/d (P<.05) on day 1 of L-NAME infusion. However, there were no significant changes in sodium balance for the overall experimental period.

Changes in Water Intake, Urinary Volume Output, and Volume Balance During L-NAME Infusion

Water intake by drinking averaged 196±60 mL/d during the control period, as shown in Fig 7. There were no significant changes in water intake for the overall L-NAME period. Urinary volume output, as shown in Fig 7, averaged 418±70 mL/d during the control period. On day 1 of L-NAME infusion, urinary volume output decreased to 142±56 mL/d (P<.05), but for the entire L-NAME period there were no significant changes in urinary volume output. Volume balance, also shown in Fig 7, increased from a control value of 0.0±46 mL/d to a value of 284±64 mL/d (P<.05) on day 1 of L-NAME infusion. However, there were no significant changes in volume balance for the entire L-NAME infusion period.

Changes in Plasma Electrolyte Concentration, Urinary Potassium Excretion, Blood Urea Nitrogen, and Plasma Creatinine During L-NAME Infusion

Plasma sodium concentration had an average value during the control period of 142.8±0.5 mmol/L. Plasma potassium had an average control value of 4.6±0.1 mmol/L. There were no significant changes in either plasma sodium or potassium concentration during L-NAME infusion. Urinary potassium excretion averaged 17.7±2.0 mmol/d during the control period and decreased to 6.7±2.2 mmol/d (P<.05) on the first day of
L-NAME. There were also moderate increases in potassium excretion on days 8 and 9 of L-NAME. Blood urea nitrogen and plasma creatinine concentrations were measured only on the first day of the recovery period in four dogs, and the values were, respectively, 0.77±0.06 mmol/L and 11.5±1.3 μmol/L. These values are very close to those measured in control dogs in another experiment in our laboratory and indicate that L-NAME did not impair renal function significantly.19

Short- and Long-term Effects of L-Arginine

Fig 8 shows the short-term effects of L-arginine on the arterial pressure response to acetylcholine, and this measurement was taken 15 minutes after the 30-minute L-arginine infusion was completed. During the control period, L-arginine caused no significant enhancement of the acetylcholine depressor effect. L-Arginine caused a significant enhancement of the acetylcholine depressor effect on days 9 through 11.

Other short-term effects of L-arginine are shown in the Table. There was a tendency for L-arginine to decrease arterial pressure acutely during L-NAME, but this effect did not reach significance. This was surprising, because L-arginine caused long-term decreases in arterial pressure during L-NAME as shown in Fig 1. However, any short-term tendency for L-arginine to decrease arterial pressure may have been opposed by short-term pressure control mechanisms such as the baroreceptors, and any baroreceptor effect may have waned chronically because of baroreceptor adaptation. The Table also shows that L-arginine administration resulted in a moderate increase in the pressor response to phenylephrine during L-NAME. However, L-arginine infusion caused no significant changes in the nitroprusside depressor response, the baroreceptor sensitivity to either nitroprusside or phenylephrine, or the acetylcholine flow response. Therefore, L-arginine caused very few short-term changes in the cardiovascular variables measured in this experiment except for the acetylcholine depressor effect.

As previously stated, L-arginine administration on days 8 through 11 of L-NAME reversed part of the increase in...
mean arterial pressure. To determine whether L-arginine caused any long-term effects on any of the other variables measured in this study, the daily values on days 8 through 11 of L-NAME (measured before the daily L-arginine infusion) were compared with the daily values on day 7, which was the last day L-NAME was administered without L-arginine. There were no significant changes in any of the following variables on days 8 through 11 of L-NAME except as noted: heart rate, change in iliac flow response to acetylcholine (compared with day 6), area under the iliac flow-time curve after acetylcholine (compared with day 6), arterial pressure responses to either nitroprusside or phenylephrine, baroreceptor reflex sensitivity to nitroprusside, baroreceptor reflex sensitivity to phenylephrine (except on day 8), SD of mean arterial pressure, SD of heart rate (except for days 10 and 11), urinary volume output (except for day 9), and volume balance (except for day 9).

Discussion
Arterial Pressure Responses to L-NAME Administration

The intravenous administration of 37.1 nmol/kg per minute of L-NAME resulted in a long-term increase in arterial pressure in conscious dogs in this experiment, confirming a major role of NO in the long-term regulation of arterial pressure. Several investigators have found that arterial pressure increases chronically in rats during NO synthesis inhibition. Gardiner et al. first demonstrated that oral L-NMMA or L-NAME increased arterial pressure for 9 hours in Brattleboro rats. Later studies by the same group showed that oral L-NMMA administration resulted in hypertension that was sustained for 7 days, and oral L-NAME administration for 5 to 6 months resulted in hypertension. Baylis et al. confirmed that oral L-NAME treatment for 2 months at 18.5 μmol/kg per day resulted in hypertension in Munich-Wistar rats. Also, Ribeiro et al. found that a much higher dose of L-NAME (222 μmol/kg per day for 4 to 6 weeks) caused arterial pressure to increase, and this hypertension could be partially reversed by L-arginine after 1 week of L-NAME. In addition, Persson et al. found that a single injection of L-NAME (61 μmol/kg IV) caused a hypertension that persisted for 24 hours. Therefore, a number of studies in rats and one study in dogs agree with our study that NO plays a long-term role in arterial pressure control.

Of major importance in studies in which NO synthesis is inhibited is the specificity of the inhibitors. Two approaches have been used to test specificity in previous studies. First, the ability of L-arginine to either prevent or reverse the hypertension is tested. Second, the ability of NO synthesis inhibition to block the effects of NO agonists such as acetylcholine is tested. In the present study, L-arginine partially reversed the hypertension, and the acetylcholine dilator and depressor effects were markedly attenuated during L-NAME. No other studies have demonstrated this reversal of acetylcholine-induced flow and depressor effects during long-term NO synthesis inhibition in animals with intact reflexes. Gardiner et al. showed that the acetylcholine depressor effect is not attenuated in conscious rats receiving L-NAME except during neurohumoral blockade; however, control rats with neurohumoral blockade without L-NAME were not run in this study. Another study in rats receiving L-NAME demonstrated that the acetylcholine-induced vasodilatation of renal, internal carotid, and common carotid beds was attenuated. Few studies outside of Gardiner's group have attempted to demonstrate a blockade of the hemodynamic effects of NO agonists during long-term NO synthesis inhibition. In the rat, it appears that the effects of acetylcholine on arterial pressure have a non-endothelium-dependent component or that reflexes severely interfere with the response. In the dogs in our study, the blockade of the depressor and dilator responses of acetylcholine was demonstrated for the first time. However, acetylcholine may cause release of vasodilator prostaglandins or other factors that may have been responsible for some of the vasodilator and vasodepressor responses. This may be the reason for the inability to achieve full inhibition of the acetylcholine flow and depressor responses during L-NAME, as seen in Figs 2 and 3.

Mechanisms of Hypertension During L-NAME

Previous experiments have shown that long-term changes in arterial pressure are associated with changes in renal function or sodium and water intake. Because overall sodium and water intake were unchanged in this experiment and arterial pressure was increased, the ability of the kidney to excrete sodium and water may have changed. Indeed, sodium output by the kidney was normal after day 1 of L-NAME infusion in the face of an increase in arterial pressure. This suggests that the long-term renal pressure-natriuresis relation may have shifted to the right along the arterial pressure axis.

Other investigators have shown that long-term L-NAME administration causes renal vasoconstriction in rats that could cause a shift in the pressure-natriuresis relation to the right. Supporting this theory are studies in rats in which renal vascular resistance increased throughout a 9-hour period of oral L-NAME administration.11 Also, oral L-NAME administration for either 4 to 6 weeks or 2 months in rats resulted in decreases in glomerular filtration rate and renal plasma flow and a large increase in renal vascular resistance.13,14 In dogs, a dose of 0.185 nmol/kg per minute of L-NAME for 3 days caused no change in arterial pressure but a decrease in glomerular filtration rate. Therefore, a number of studies lend support to the theory that L-NAME causes changes in renal function that could cause a shift in the pressure-natriuresis relation.

Some investigators have shown that sodium output increases after acute administration of L-NAME. Baylis et al. infused a 37 μmol/kg IV bolus of L-NAME into conscious rats and found an increase in urinary sodium excretion. Johnson and Freeman also found a natriuresis in anesthetized rats after a 297 μmol/kg IV bolus of L-NAME. In both cases, arterial pressure increased abruptly after L-NAME, and in one study the natriuresis was prevented by maintaining renal perfusion pressure at its normal value. Salazar et al. recently found that intravenous infusion of 0.185 nmol/kg per minute of L-NAME caused no change in mean arterial pressure but a decrease in urine sodium excretion and water volume. According to this study, the renal effects of NO synthesis inhibition occur at lower doses of L-NAME than do the arterial pressure effects. In the present experiment, the dogs received only 53.4 μmol/kg per day of L-NAME, and urinary sodium excretion decreased.
only on the first day. Urinary potassium excretion also decreased on the first day of L-NAME. Thus, it is unlikely that increases in plasma aldosterone concentration were responsible for the decreased sodium excretion. It appears that whether excess natriuresis occurs depends on how fast the initial increase in renal perfusion pressure occurs because of the caudal tubal perfusion. L-NAME has been shown to cause sodium retention in conscious dogs.17

The overall sodium and water balances were unchanged during the L-NAME period. This suggests that fluid volumes were unchanged and that hypertension associated with NO synthesis blockade is not a volume-dependent type of hypertension. In fact, others have shown that L-NAME increases vascular resistance in the renal, mesenteric, and hindquarter vascular beds.20 In addition, L-NAME infusion has been associated with increases in total peripheral resistance and decreases in cardiac output.15,24 Therefore, L-NAME infusion may produce a vasoconstrictor type of hypertension.

Another factor that may cause part of the increase in arterial pressure during L-NAME is an increase in plasma angiotensin II concentration. Salazar et al17 found that plasma renin activity increased during L-NAME in dogs. Also, Ribeiro et al13 found that plasma renin activity increased in rats on long-term oral L-NAME and that angiotensin II antagonism with losartan attenuated the increase in arterial pressure. However, in preliminary studies, Samsell et al25 found that acute losartan administration to rats on long-term oral L-NAME did not cause a decrease in arterial pressure. Whether angiotensin caused part of the increase in arterial pressure in the present experiment is not clear.

Another factor that could have caused part of the increase in arterial pressure during L-NAME in the present study is an increase in sympathetic output. Sakuma et al26 recently showed that renal sympathetic nerve activity increased during acute L-NAME. However, Samsell et al25 in preliminary studies found that arterial pressure increased 36% in rats on long-term L-NAME and 37% in rats on long-term L-NAME plus short-term prazosin. Therefore, whether increased sympathetic output contributes to the increase in arterial pressure in the present study is not clear.

The hypertensive response to L-NAME infusion could have been caused by directly decreasing the vasodilator effects of NO on the vasculature, or other hypertensive factors, normally attenuated by NO, may have contributed to the increase in arterial pressure. Part of the increase in arterial pressure during NO synthesis blockade could have been caused by an enhanced response of the vascular smooth muscle to vasoconstrictors such as phenylephrine; however, there was no significant increase in the phenylephrine pressor response after the second day of L-NAME in this study. Second, part of the increase in arterial pressure could have been caused by a diminished vasodilator response of the vascular smooth muscle to endogenous dilators or endogenous NO donors. However, when the exogenous NO donor nitroprusside was given, the opposite effect occurred during L-NAME administration, and the nitroprusside hypertensive response actually increased. Because NO release decreases during competitive inhibition of NO synthase, the additional decrease in arterial pressure during nitroprusside infusion could be attributed to a supersensitivity phenomenon.27 This putative supersensitivity is further evidence that NO synthesis was blocked. Another factor that could have caused part of the increase in arterial pressure in this experiment is the release of endothelium-derived contracting factors.28 However, our data do not directly address this possibility. Nevertheless, the data in this experiment may suggest that the increase in arterial pressure during the L-NAME state arterial pressure increase during L-NAME infusions is caused by changes in vascular smooth muscle responsiveness to vasoactive substances such as phenylephrine or sodium nitroprusside.

Several studies have indicated that the baroreceptors may be affected by endothelium-derived substances, and this could contribute to arterial pressure and heart rate changes during L-NAME. Exogenous prostaglandin I2 increased baroreceptor activity during increases in carotid sinus pressure in normotensive29,30 and hypertensive31 rabbits. Balloon denudation of the carotid sinus, which would deplete this area of NO and prostaglandin I2, caused a decrease in baroreceptor activity.30 However, the effects of NO on baroreceptor reflex sensitivity are not clear. The overall cardiovascular effects of changes in baroreceptor reflex activity are dependent on local changes at the baroreceptors as well as the integration of the baroreceptor signal in the central nervous system, which may be affected by NO. Nevertheless, in our experiment, L-NAME caused significant decreases in the heart rate component of baroreceptor reflex sensitivity to nitroprusside. However, the variability of heart rate, as measured by the 24-hour SD, was significantly decreased throughout L-NAME. In a recent study by Persson et al19 in which nitro-l-arginine was injected as a bolus into conscious dogs, the 24-hour SD of heart rate also tended to decrease, but the change did not reach significance. Also, in this study, arterial pressure increased for 24 hours after short-term L-NAME in the dogs, and the SD of arterial pressure increased significantly from 9.5±0.4 mm Hg during control to 11.7±1.1 mm Hg during L-NAME.16 The control value of SD of arterial pressure in their study was very close to the control SD in our study of 10.0±0.8 mm Hg, but our SD during L-NAME increased even more to 13.1±1.4 mm Hg. The variability of arterial pressure and heart rate reflects not only changes in baroreceptor reflex function as we have previously shown in sinoaortic-denervated dogs32 but also activity and postural changes in dogs.16 In the present study during L-NAME, the dogs were noticeably quieter, which may partially explain the decrease in heart rate variability. The variability of arterial pressure overcame any tendency to decrease because of less physical activity and showed a net increase, which is consistent with a decrease in baroreceptor reflex sensitivity. This suggests that the mechanisms controlling arterial pressure during L-NAME were less effective than during the control period.

A number of cardiovascular variables measured in this experiment were slow to return to control after L-NAME was discontinued. However, arterial pressure was not significantly different from control after the second day of recovery from L-NAME. Baylis et al14 found that arterial pressure returned to control 48 hours after L-NAME treatment was stopped, and these rats had received L-NAME for 6 months. However, Ribeiro et al13 found that arterial pressure was still elevated in
vasodepressor effects were strikingly decreased during National Heart, Lung, and Blood Institute. unchanged for the overall L-NAME period, indicating only on the first day of NO synthesis blockade and were L-NAME. Sodium and water balances were positive L-NAME into conscious dogs. This hypertension was ion can be sustained for long periods of time during results in prolonged NO synthesis inhibition, but L-arginine may help reverse this inhibition. In conclusion, our results demonstrate that hypertension can be sustained for long periods of time during NO synthesis blockade with intravenous infusion of L-NAME into conscious dogs. This hypertension was associated with an increase in arterial pressure variability and only a transient change in the phenylephrine pressor sensitivity. L-Arginine partially reversed this hypertension, and the acetylcholine vasodilator and vasopressor effects were strikingly decreased during L-NAME. Sodium and water balances were positive only on the first day of NO synthesis blockade and were unchanged for the overall L-NAME period, indicating that this increase in pressure is not associated with volume retention. The data suggest that there is a basal release of NO by vascular endothelial cells that affects long-term arterial pressure regulation in dogs.

Acknowledgments
This research was supported by grant HL-11678 from the National Heart, Lung, and Blood Institute.

We would like to thank Susie Araysi and Ivaldele Heidke for typing the manuscript.

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*Hypertension*. 1993;22:40-48
doi: 10.1161/01.HYP.22.1.40

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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