Sustained Hypertension Induced by Orally Administered Nitro-L-Arginine

Jamie Dananberg, Richard S. Sider, and Roger J. Grekin

To study the hemodynamic and metabolic effects of chronic inhibition of endothelium-derived nitric oxide, we treated conscious rats with an oral solution of N^o-nitro-L-arginine (LNA), an inhibitor of nitric oxide production by endothelial cells. After 3 days of treatment with 2.74 mM LNA, rats had higher blood pressures (136±5 versus 113±3 mm Hg, p<0.0005) than did the control animals. This effect was maintained through 7 days of treatment (142±6 versus 109±4 mm Hg, p<0.0005) and in three animals for 35 days (167±7 mm Hg). The blood pressure rise was dose dependent. The hypertensive effect of oral LNA was not enhanced by the administration of 20 mg intraperitoneal LNA and was prevented by pretreatment with L-arginine, although L-arginine also caused a transient but significant increase in urinary sodium excretion. When LNA treatment was discontinued, blood pressure fell gradually, with an effective biological half-life of 4.2 days. Metabolic balance studies did not identify differences in sodium or potassium balance between treated and control animals. Plasma renin activity was lower in LNA-treated animals, and aldosterone concentrations tended to be lower. In contrast, atrial natriuretic factor levels and serum electrolyte concentrations were unchanged after 7 days of treatment with LNA. These data support the premise that endothelium-derived nitric oxide plays an important role in basal hemodynamic homeostasis. Oral administration of LNA may serve as a model of chronic nitric oxide-deficient hypertension and allow for the future study of endothelium dependence in hypertension. (Hypertension 1993;21:359-363)

KEY WORDS • nitric oxide • arginine • endothelium-derived relaxing factor

Nitric oxide (NO) generation by endothelial cells appears to account for a major component of endothelium-dependent relaxing factor activity. NO synthase, the enzyme responsible for NO generation, has recently been cloned from brain tissue. The enzyme uses arginine as its sole substrate, and several analogues of arginine have been shown to be specific competitive inhibitors of NO production. With the introduction of these agents, the importance of endogenous NO production in basal regulation of blood pressure in vivo has come under increasing investigation. Rees et al reported that intravenously administered N^o-monomethyl L-arginine (L-NMMA) raised blood pressure in anesthetized rabbits, and Aisaka et al found similar results in anesthetized guinea pigs. In the rat, blood pressure increases after intravenous infusion of L-NMMA and N^o-nitro-L-arginine-methyl ester. In humans, L-NMMA infusion into the brachial artery decreases forearm blood flow. Hecker et al have shown that another arginine analogue, N^o-nitro-L-arginine (LNA), when administered intravenously, is more potent than L-NMMA in raising blood pressure in the anesthetized rat.

No studies to date have investigated the effects of NO synthase inhibitors on the intact, conscious animal. Furthermore, all prior work has examined only the intravenous administration of these compounds. In this study, we sought to determine the endocrine, hemodynamic, and metabolic effects of LNA when given orally to otherwise normal rats.

Methods

Metabolic Balance and Hormonal Studies

Twelve male Sprague-Dawley outbred rats (Charles River, Portage, Mich.) with initial weights of 300–325 g were studied in metabolic cages for 10 days. For 3 days, all rats were given free access to water. Over the next 7 days, six rats were given water with LNA added to a concentration of 2.74 mM in place of drinking water. Six control animals received tap water throughout the study. All rats had free access to rat chow during the study period (Purina Mills, Inc., St. Louis, Mo.; 0.34% sodium, 1.50% potassium). The three baseline days were termed days −3 through −1 and the intervention period as days 1 through 7. Based on water consumption and animal weights, the average dosage of LNA in the treated animals was 52.56 mg/kg per 24 hours.

Blood pressure and pulse were measured by the indirect tail-cuff technique on days −5, 3, and 7. Before measurement, the rats were warmed with a heating pad for 3 minutes. Ten to 20 measurements were taken 30 seconds apart with a Programmed Electro-sphygmoma...
nometer (model PE-300, Narco BioSystems, Austin, Tex.) calibrated with a mercury manometer. The results were recorded on a model 7D polygraph (Grass Instrument Co., Quincy, Mass.). The mean of the last five measurements was used as the blood pressure. Body weight was also measured on these days. The validity of the use of the tail-cuff method of blood pressure determination in LNA-treated animals was determined by performing a parallel experiment in which blood pressure was assessed by carotid artery catheterization in eight control animals and in nine animals treated with 2.54 mM LNA.

From day -3 to day 7, daily water and food intake, urine volume, and urinary concentrations of sodium, chloride, and potassium were measured. Electrolytes were measured by a Synchron CX 3 System (Beckman Instruments, Inc., Brea, Calif.). Two days after the animals were removed from the metabolic cages but still drinking either water or LNA plus water, the rats were killed by decapitation. Trunk blood was saved for radio-immunoassay of aldosterone,"""" plasma renin activity,"""" and atrial natriuretic factor."

**Dose Response**

To determine the relation between the ingested dose of LNA and pressor response, we studied six rats during the administration of increasing concentrations of LNA. Animals were given tap water for 1 week before study, and blood pressure and heart rate were determined by the tail-cuff method at the end of the control period. During the next six days, LNA was added to the drinking water; doses were then increased in a stepwise fashion. Rats received 0.25 mM LNA for 2 days, 0.91 mM for 2 days, and 2.74 mM for 2 days. Blood pressure and heart rate were measured after 48 hours of each treatment. These rats were maintained on 2.74 mM LNA for an additional 12 days and then were switched to tap water. Blood pressure and heart rate were determined on the last day of LNA treatment and sequentially after its discontinuation.

**Oral Versus Parenteral Treatment**

Five rats were given a large dose of oral LNA, 9.1 mM, for a 5-day period so that we could determine whether oral LNA could block the pressor response to parenterally administered LNA. After 5 days of treatment, blood pressure and heart rate were determined, and a single intraperitoneal injection was made of 2 mL LNA, 45.5 mM, in 5% dextrose in water (total dose, 0.091 mmol). Eight control rats also received a single intraperitoneal injection of LNA. Blood pressure was measured every 5 minutes from 20 to 40 minutes after injection.

All protocols involving animals followed animal care guidelines established by the Subcommittee on Animal Use at the VA Medical Center and the Department of Lab Animal Medicine at the University of Michigan.

**Prevention of Hypertensive Response by L-Arginine**

Twelve rats were given oral LNA, 2.74 mM, over a 2-day period. Six of the animals were pretreated with 274 mM L-arginine 2 days before and during LNA treatment. After the treatment period, blood pressure in both groups was measured by the tail-cuff method. The effect of these treatments on urinary sodium excretion was also evaluated in six animals housed in metabolic cages. Basal collections were made during the first 2 days, after which the animals were treated with 274 mM L-arginine for 2 days and then with 2.74 mM LNA and L-arginine for 2 days. Urine was collected on a daily basis, and electrolytes were measured as described above. Food and water intake and body weight were assessed daily.

**Results**

The changes seen in blood pressure and heart rate after 3 and 7 days of treatment with 2.74 mM LNA as the sole drinking source are shown in Figure 1. Blood...
pressure and pulse were not different between the two groups during the baseline measurement period. After 3 days of treatment, animals given LNA had significantly higher blood pressures (136±5 versus 113±3 mm Hg, \( p < 0.0005 \)) and lower heart rates (323±7 versus 365±13 beats per minute, \( p < 0.01 \)) than did animals given tap water. The elevation in blood pressure was maintained at 7 days (142±6 versus 109±4 mm Hg, \( p < 0.0005 \)); however, differences in heart rate at this time were not statistically significant. The validity of the tail-cuff method of blood pressure determination was determined in catheterized animals. In this experiment, LNA-treated animals had significantly higher mean arterial blood pressures than did control animals as measured by carotid artery catheters (156±6 versus 120±9 mm Hg, \( p < 0.001 \)).

The corresponding changes in electrolyte balance during this same time period are illustrated in Figure 2. These data indicate that during the intervention phase of the protocol, sodium and potassium balance did not change significantly between those animals treated with LNA and controls. There was a significant difference in urinary potassium excretion during the basal period between the two groups (6.2±0.4 meq/24 hours, LNA treatment, versus 4.7±0.3 meq/24 hours, control; \( p < 0.01 \)) and small differences in sodium and chloride excretion, which approached but did not reach statistical significance. These changes are explained by a difference in intake between the two groups. Control rats consumed significantly less food during the baseline period (19±0.9 g, control animals, versus 22±0.9 g, LNA animals; \( p < 0.005 \)). This difference, along with water consumption and total urine output, is shown in Figure 3. Except for food intake during the baseline period, no significant differences between the two groups were noted.

The metabolic effects of LNA administered orally are summarized in Table 1. Serum electrolytes were not different between study and control rats at the end of the protocol after 12 days of LNA treatment. Plasma renin activity was significantly lower in LNA-treated animals. Differences in aldosterone levels between the two groups approached statistical significance. Atrial natriuretic factor levels were not different between the groups.

**Table 1. Metabolic Effects of Oral N^\text{\textsuperscript{o}}\text{-Nitro-L-Arginine}**

<table>
<thead>
<tr>
<th>Factor measured</th>
<th>Control</th>
<th>LNA-treated</th>
<th>( n )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (meq/L)</td>
<td>142±1</td>
<td>142±1</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Chloride (meq/L)</td>
<td>108±2</td>
<td>108±1</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium (meq/L)</td>
<td>8.0±0.3</td>
<td>8.0±0.5</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Atrial natriuretic factor (pmol/L)</td>
<td>57±16</td>
<td>30±6</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>Renin activity (ng/mL per hour)</td>
<td>20.2±1.9</td>
<td>11.1±1.3</td>
<td>12</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Aldosterone (pg/mL)</td>
<td>104±24</td>
<td>55±9</td>
<td>12</td>
<td>0.073</td>
</tr>
</tbody>
</table>

LNA, N^\text{\textsuperscript{o}}\text{-nitro-arginine.}
sures analysis of variance.

minutes after intraperitoneal injection of N\textsuperscript{\textdegree}nitro-L-arginine (LNA), 0.091 mmol, in animals given oral LNA, 9.1 mM, in 8% sucrose and 0.01 M HCl (a) as drinking source for 6 days, and in control animals (●). *p=0.0011, LNA vs. control, **p<0.0001, postinjection vs. preinjection, by repeated-measures analysis of variance.

The results of the dose–response study are shown in Figure 4. At increasing doses, there is a step-wise increase in the mean blood pressure as measure by the indirect tail-cuff method (118.8±3.0, 123.7±0.6, 136.8±3.6, and 148.0±5.6 mm Hg) and a corresponding decrease in the heart rate. When LNA was removed from the drinking source, the blood pressure fell linearly. After 5 days, blood pressure was no longer significantly different from baseline levels. The calculated effective biological half-life of orally administered LNA was 4.2 days. Similar changes were noted in heart rate.

The ability of oral LNA to block the hypertensive response to injected LNA is shown in Figure 5. In control animals, intraperitoneal injection of 0.091 mmol LNA caused a rapid increase in blood pressure from 121±2 to 166±3 mm Hg (p=0.0001). In contrast, mean blood pressure did not change in animals pretreated with oral 2.74 mM LNA. Animals pretreated with oral LNA had higher mean blood pressures than control animals (141±7 versus 121±3 mm Hg, p=0.0011). After injection, however, blood pressure rose in control animals, exceeding the rise seen in the group pretreated with orally administered LNA (166±3 versus 137±7 mm Hg, p<0.0001). Three rats in the oral treatment group were maintained on oral LNA for 35 days. In these animals, the blood pressures at this time were 172, 153, and 175 mm Hg (mean, 167±7 mm Hg).

To assess the specificity of the hypertensive response to oral LNA, we evaluated the ability of L-arginine pretreatment to block this response. These data are shown in Figure 6. Animals given 274 mM L-arginine for 2 days had blood pressures similar to those of control animals (124±4 versus 123±4 mm Hg). After administration of 2.74 mM oral LNA to both groups for 2 days, control animals had a marked increase in blood pressure (153±6 mm Hg, p<0.001, compared with basal blood pressure). In contrast, animals pretreated with L-arginine had no change in blood pressure (123±6 mm Hg). To assess the specificity of the inhibition of hypertension by L-arginine, we evaluated its effect on sodium excretion. These results are depicted in Figure 7 and indicate that L-arginine caused a significant change in sodium balance compared with control days. After 24 hours of L-arginine treatment, mean urinary sodium balance was more negative by 2.05 mmol sodium per 24 hours (p<0.0001).

Discussion

The use of analogues of L-arginine that specifically inhibit NO production in the intact animal has provided evidence that endothelial production of NO is an important regulator of hemodynamic homeostasis. Several studies have demonstrated an increase in mean systolic pressure after intravenous L-NMMA infusions in anesthetized rabbits\textsuperscript{1} and rats.\textsuperscript{12} Brain microvessels have been shown to be responsive to L-NMMA in the mouse.\textsuperscript{13} Umans et al\textsuperscript{14} also found increases in mean arterial blood pressure in chronically instrumented conscious rats after intravenous administration of LNA. In humans, Vallance et al\textsuperscript{15} showed that brachial artery infusions of L-NMMA significantly reduced forearm blood flow and attenuated the depressor effect of acetylcholine. These data, taken together, suggest that NO production plays an important role in determining basal vascular tone. However, these studies do not provide information about the long-term effects of arginine analogues on blood pressure.

The present work was undertaken in part to establish a model of chronic NO-deficient hypertension. Although no information is available on the absorption of orally administered LNA, we speculated that it would...
be readily absorbed through the gastrointestinal tract in a manner similar to arginine itself. We were able to show a significant, long-term effect of orally administered LNA on blood pressure in the conscious rat. Furthermore, these effects persisted as long as LNA was included in the drinking water; maintenance of the hypertensive state was demonstrated in these animals for 35 days. Additionally, we were able to show that the blood pressure response was dose related. Intravenously administered L-NMMA has also been shown to elicit dose-dependent changes in blood pressure, brain microvessel size, and forearm blood flow. Intraperitoneal LNA did not add to the hypertensive effect of oral LNA treatment administered for 6 days, suggesting that orally administered LNA given chronically completely suppressed NO production. This study also found that the blood pressure response to oral LNA was inhibited by pretreatment with L-arginine. Although L-arginine can reverse the inhibition of NO synthase by arginine analogues, its use in vivo is complicated by its effects on renal function. High-dose L-arginine increases renal blood flow. This study found that L-arginine significantly increases urinary sodium excretion. It is possible that L-arginine inhibition of the increase in blood pressure induced by LNA may in part be due to its reduction in blood volume. However, L-arginine alone did not alter blood pressure significantly. These data, taken together, suggest that oral LNA causes inhibition of endogenous NO production. Additional studies examining the role of volume status on LNA-induced increases in blood pressure are required to assess the importance of L-arginine inhibition of this response.

Metabolic studies revealed no difference in sodium balance between LNA-treated and control animals at any time. Although several studies have shown that endothelial-dependent responses are impaired in salt-sensitive forms of hypertension, the results of this study suggest that inhibition of endothelium-derived NO itself does not lead to sodium retention. The reduction in plasma renin activity and aldosterone is likely to be related to the inhibitory effect of increased blood pressure on renin release.

These data strongly support the hypothesis that endothelial production of NO is an important basal regulator of blood pressure homeostasis. Furthermore, this study illustrates a model of chronic hypertension based on inhibition of endothelial production of NO. This model could be useful in the future study of endothelium dependence in hypertension.

References
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