Control of Regional Blood Flow by Endothelium-Derived Nitric Oxide

Sheila M. Gardiner, Alix M. Compton, Terence Bennett, Richard M.J. Palmer, and Salvador Moncada

The regional hemodynamic consequences of inhibiting vascular endothelial nitric oxide generation with \( N^\omega \)-monomethyl-L-arginine (L-NMMA) were studied in conscious Long-Evans rats. Experiments were carried out in groups of chronically instrumented rats with intravascular catheters and pulsed Doppler probes to monitor regional blood flow. L-NMMA (0.3-300 mg/kg) caused a dose-dependent, long-lasting (5-90 minutes), and enantiomerically specific increase in mean blood pressure and also caused bradycardia. The increase in blood pressure was accompanied by a dose-dependent and long-lasting vasoconstriction in the internal carotid, mesenteric, renal, and hindquarters vascular beds that could be attenuated, in a concentration-dependent manner, by L-arginine but not by D-arginine. In contrast, L-arginine did not affect the pressor or vasoconstrictor effects of vasopressin. These results indicate that nitric oxide production by vascular endothelial cells contributes to the maintenance of blood pressure and to the control of the resting tone of different vascular beds in the conscious rat. (Hypertension 1990;15:486-492)

There is now compelling evidence that a vasodilator produced by endothelial cells is nitric oxide (NO) (for review, see Reference 1). This NO is formed from L-arginine by an enzyme that can be inhibited in vitro in an enantiomerically specific manner by \( N^\omega \)-monomethyl-L-arginine (L-NMMA). L-NMMA causes a dose-dependent increase in mean systemic arterial blood pressure in anesthetized rabbits, accompanied by a reduction in ex vivo release of NO; these effects are not seen with D-NMMA. Furthermore, the elevation of blood pressure and the reduction in ex vivo release of NO are both reversed by L-arginine but not D-arginine. These observations indicate that, in vivo, the formation of NO by vascular endothelial cells plays an important role in the control of blood pressure. In the present study, we have investigated this role further by examining the regional hemodynamic consequences of inhibiting vascular endothelial cell production of NO in conscious rats.

Methods

Male Long-Evans rats (350-400 g) were anesthetized (sodium methohexitone, 60 mg/kg i.p., supplemented as required) and pulsed Doppler probes were sutured around the left renal and superior mesenteric arteries and the distal abdominal aorta below the level of the ileocecal artery (i.e., to monitor blood flow to the hindquarters). Other rats had a probe sutured around one common carotid artery after the external carotid artery on the same side had been ligated; this made it possible to monitor internal carotid blood flow (see below). Probe wires were fed subcutaneously and emerged at the back of the neck where they were secured with a ligature. After 7-14 days, rats were briefly reanesthetized (sodium methohexitone, 40 mg/kg i.p.), and catheters were implanted in the jugular vein for drug administration and in the distal abdominal aorta (via the ventral caudal artery) for monitoring mean arterial blood pressure and instantaneous heart rate. The catheters were fed subcutaneously to exit at the same point as the probe wires. The latter were soldered into a microconnector (Microtech Inc., Boothwyn, Pennsylvania) that was clamped to a harness worn by the rat. The catheters ran through a flexible spring connected to the harness. Experiments were started at least 24 hours later, when rats were conscious and unrestrained and housed in their home cages with free access to food and water.

The experimental protocols ran over 2-3 days and involved continuous measurements (between 6:30 AM and 5:00 PM) of mean blood pressure, heart rate, and renal, mesenteric, and hindquarters or internal carotid Doppler shift signals. Although the Doppler
shift signal is not a direct measure of volume flow, percentage changes in this signal under the conditions of our experiments are a reliable indication of changes in volume flow. Changes in vascular conductance were calculated from mean blood pressure and the mean Doppler shift signal, but the phasic Doppler shift signal was monitored continuously to ensure that it was of acceptable quality (signal:noise >20:1). The Doppler shift signals were monitored with a flowmeter (VF-1 system with HVPD 20 modules, Crystal Biotech, Holliston, Massachusetts), and all variables were recorded on a Gould ES1000 electrostatic recorder (Gould Electronics Ltd., Essex, UK).

**Experimental Protocols**

**Experiment 1: Dose responses to L-NMMA acetate.**
Rats with renal, mesenteric, and hindquarters probes (n=8) and rats with internal carotid probes (n=8) were given increasing intravenous doses of L-NMMA acetate (0.3, 1.0, 10, 30, 100, and 300 mg/kg), separated by at least 45 minutes. The three highest doses of L-NMMA were given to a different group of rats with renal, mesenteric, and hindquarters probes (n=8). Two rats were also given D-NMMA (100 mg/kg).

**Experiment 2: Dose responses to L-arginine acetate.**
The rats that were given L-NMMA at a dose of 100 mg/kg in experiment 1 were given the same dose of (n=8) and rats with internal carotid probes (n=8) were given increasing intravenous doses of L-NMMA acetate (0.3, 1.0, 10, 30, 100, and 300 mg/kg), separated by at least 45 minutes. The three highest doses of L-NMMA were given to a different group of rats with renal, mesenteric, and hindquarters probes (n=8). Two rats were also given D-NMMA (100 mg/kg).

FIGURE 1. Line graphs showing cardiovascu-
lar changes after administration of N^6-
monomethyl-L-arginine (L-NMMA) in con-
scious Long-Evans rats bearing (panel A) renal, mesenteric, and hindquarters flow probes (n=8) and (panel B) internal carotid probes (n=8). L-NMMA dose (mg/kg): 0.3, •-•; 1.0, ○-○; 3.0, ■■; 10, ■■■; 30, ▲▲. Values are mean±SEM. BP, blood pressure.

FIGURE 2. Line graphs showing changes in Doppler shift after administration of increasing doses of N^6-monomethyl-L-arginine (L-NMMA) in conscious Long-Evans rats with renal, mesenteric, and hindquarters probes, or internal carotid probes (n=8 for each group). L-NMMA dose (mg/kg): 0.3, •-•; 1.0, ○-○; 3.0, ■■; 10, ■■■; and 30, ▲▲. Values are mean±SEM.

FIGURE 3. Line graphs showing changes in vascular con-
ductance after administration of increasing doses of N^6-
monomethyl-L-arginine (L-NMMA) in conscious Long-Evans rats with renal, mesenteric, and hindquarters probes, or internal carotid probes (n=8 for each group). L-NMMA dose (mg/kg): 0.3, •-•; 1.0, ○-○; 3.0, ■■; 10, ■■■; 30, ▲▲. Values are mean±SEM.
FIGURE 4. Line graphs showing changes in mean blood pressure (BP), Doppler shift, and vascular conductance measured 5 minutes after administration of increasing doses of N\textsuperscript{o}-monomethyl-L-arginine (L-NMMA) in conscious Long-Evans rats with renal, mesenteric, and hindquarters probes, or internal carotid probes (n=8 for each group). Mean BP responses: ○, rats with renal, mesenteric, and hindquarters probes; ●, rats with internal carotid probes. Doppler shift or conductance responses: ●, renal; ▲, mesenteric; ■, hindquarters; ◊, internal carotid. Values are mean±SEM.

L-NMMA and, starting 10 minutes later, received increasing intravenous doses of L-arginine acetate (35, 100, and 300 mg/kg) at 10-minute intervals. The two exposures to L-NMMA were at least 24 hours apart. These rats also received L-arginine acetate alone at a dose of 100 mg/kg. A separate group of rats (n=8) was given L-arginine acetate alone at 300 mg/kg.

Experiment 3: Dose responses to \(\text{D-arginine acetate.}\) Another group of rats with renal, mesenteric, and hindquarters probes (n=8) was given L-NMMA (100 mg/kg) on two occasions (separated by 24 hours). On one occasion, starting 10 minutes after administration of L-NMMA, D-arginine acetate was administered intravenously (35, 100, and 300 mg/kg) at 10-minute intervals. These rats also received D-arginine acetate alone at a dose of 100 mg/kg.

Experiment 4: Dose response to L-arginine hydrochloride. The rats involved in the protocol for experiment 3 were given, on another experimental day, L-NMMA at a dose of 100 mg/kg followed 10 minutes later by increasing doses of L-arginine hydrochloride (35, 100, and 300 mg/kg) at 10-minute intervals. These rats also received L-arginine hydrochloride and L-lysine acetate alone (each at 100 mg/kg).

The rats that had received 300 mg/kg L-arginine acetate (experiment 2) also received 300 mg/kg L-arginine hydrochloride at least 24 hours later.

Experiment 5: Effects of L-arginine hydrochloride on the responses to vasopressin. The rats that were used to assess the effects of L-arginine acetate or hydrochloride (experiment 4) also received L-NMMA (100 mg/kg) or vasopressin (36–90 pmol/hr) on separate days followed 10 minutes later by L-arginine hydrochloride (300 mg/kg).

The mean blood pressure and heart rate of the 40 rats used in this study were 103±2 mm Hg and 352±11 beats/min, respectively, and there were no significant differences between any of the groups of rats used in the five experiments. In all experimental protocols drugs were dissolved in physiological saline at pH 7 (see below) and injected intravenously in volumes of 0.1 ml. All injections were given intravenously in a bolus of 0.1–0.2 ml (catheter dead space 0.1 ml), flushed in with 0.1 ml isotonic saline. Administration of vehicle alone had no significant cardiovascular effects.

Chemicals

All compounds used (excluding vasopressin) were obtained from Wellcome Research Laboratories (Langley Court, Beckenham, UK) and where necessary synthesized as previously described. Solutions
Figure 6. Line graphs showing changes in Doppler shift after administration of N^ω^-monomethyl-L-arginine (L-NMMA) (100 mg/kg) alone (○-○) or L-NMMA followed by increasing doses of L-arginine acetate (L-Arg) (●-●) in the same groups of conscious Long-Evans rats with either renal, mesenteric, and hindquarters probes or internal carotid probes (n=8 for each group). Values are mean±SEM.

of L-arginine hydrochloride were adjusted to pH 7 with sodium hydroxide so that all solutions used had a similar pH. Vasopressin ([Arg^8] vasopressin, Bachem, Saffron Walden, Essex, UK) was dissolved in physiological saline containing 1% bovine serum albumin and was infused in 0.3 ml/hr.

Statistics
Data were subjected to Friedman's test, Wilcoxon's rank sum test, or the Mann-Whitney U test as appropriate, and p<0.05 was taken as indicating statistical significance.

Results
Dose Responses to L-NMMA
L-NMMA (0.3–30 mg/kg) induced a dose-dependent increase in mean blood pressure and a decrease in heart rate that were maximal within 2 minutes and had generally stabilized by 5 minutes. The effects were not significantly different between those rats with renal, mesenteric, and hindquarters probes and those with internal carotid probes (Figure 1). L-NMMA (0.3–30 mg/kg) also induced, within 2 minutes, a dose-dependent decrease in blood flow (Figure 2) and in vascular conductance (Figure 3) in the renal, mesenteric, and internal carotid vascular beds that was significant at doses of 1 mg/kg and above at this time.

There were some differences in the initial response of the vascular beds. There was a significant vasoconstriction in the mesenteric vascular bed 1 minute after administration of 0.3 mg/kg L-NMMA. In the hindquarters, there was an early (1–2 minute), significant vasodilatation induced by 0.3 and 1 mg/kg L-NMMA, although at later times and with higher doses there was only vasoconstriction (Figures 2 and 3).

Five minutes after administration of L-NMMA (0.3–300 mg/kg), all vascular beds showed similar
degrees of vasoconstriction, apart from a slightly reduced maximum response to the higher doses of L-NMMA in the renal vascular bed (Figure 4). The duration of the effects of L-NMMA (0.3–300 mg/kg) on mean blood pressure, heart rate, and regional vascular conductances was also dose-dependent, lasting between 5 and 90 minutes. D-NMMA acetate (100 mg/kg) caused no pressor or regional vasoconstrictor effects; there was a transient mesenteric vasodilatation that was probably attributable to the acetate moiety (see below).

**Attenuation by L-Arginine Acetate of Effects of L-NMMA**

Administration of L-NMMA (100 mg/kg) caused a sustained increase in mean blood pressure, decrease in heart rate, and regional vasoconstrictions (Figures 5–7) that returned slowly toward control levels over a period of 90 minutes. L-Arginine acetate (35–300 mg/kg) caused a significant dose-dependent attenuation of the effects of L-NMMA on mean blood pressure and heart rate, although mean blood pressure was still significantly elevated 10 minutes after administration of 300 mg/kg (Figure 5). L-Arginine acetate also attenuated the vasoconstrictor effects of L-NMMA. There were, however, some differences in the degree of attenuation that was achieved (Figures 6 and 7). Thus, 10 minutes after administration of L-arginine acetate (300 mg/kg), the renal vascular conductance was not significantly different from the basal level, whereas mesenteric, internal carotid, and hindquarters vascular conductances were still 10±3, 31±4, and 33±4% below baseline, respectively (p<0.05 for all).

Immediately after administration of L-arginine acetate, the mesenteric vascular bed showed a significant, dose-dependent hyperemia and vasodilatation (Figures 6 and 7) that rapidly waned, although flow and conductance remained higher than before administration of L-arginine acetate. D-Arginine acetate did not attenuate the cardiovascular effects of L-NMMA, although a transient mesenteric vasodilatation was observed (Figure 8). A similar mesenteric response at 1 minute was seen when L-arginine acetate, L-lysine acetate, and D-arginine acetate, but not L-arginine hydrochloride, were administered alone at doses of 100 mg/kg (Table 1). The mesenteric vasodilator effects of L-arginine acetate increased with dose, but even at a dose of 300 mg/kg, L-arginine hydrochloride did not affect mesenteric

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**Table 1. Cardiovascular Changes 1 Minute After Administration of Bolus Doses of Arginine or Lysine in Conscious Long-Evans Rats**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Heart rate (beats/min)</th>
<th>MBP (mm Hg)</th>
<th>Flow (%)</th>
<th>Conductance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>Renal</td>
<td>Renal</td>
</tr>
<tr>
<td>L-Arginine acetate (100 mg/kg) (n=7)</td>
<td>33±11*</td>
<td>12±3*</td>
<td>9±3*</td>
<td>-15±5*</td>
</tr>
<tr>
<td>L-Arginine acetate (300 mg/kg) (n=8)</td>
<td>29±4*</td>
<td>7±2*</td>
<td>13±3*</td>
<td>73±4*</td>
</tr>
<tr>
<td>L-Arginine hydrochloride (100 mg/kg) (n=7)</td>
<td>23±7*</td>
<td>2±1</td>
<td>5±1*</td>
<td>7±3</td>
</tr>
<tr>
<td>L-Arginine hydrochloride (300 mg/kg) (n=8)</td>
<td>1±8</td>
<td>2±2</td>
<td>6±1*</td>
<td>8±3</td>
</tr>
<tr>
<td>D-Arginine acetate (100 mg/kg) (n=9)</td>
<td>24±3*</td>
<td>14±1*</td>
<td>3±4</td>
<td>49±6*</td>
</tr>
<tr>
<td>L-Lysine acetate (100 mg/kg) (n=9)</td>
<td>28±8*</td>
<td>10±3</td>
<td>10±2*</td>
<td>41±6*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *p<0.05 vs. baseline (Wilcoxon's rank sum test). A, Change from baseline; MBP, mean blood pressure; n, number of rats in each group.

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**Table 2. Effects of L-Arginine Hydrochloride on Cardiovascular Responses to N°-Monomethyl-L-arginine or Vasopressin in the Same Conscious Long-Evans Rats (n=8).**

<table>
<thead>
<tr>
<th>Hemodynamic measurements</th>
<th>L-NMMA</th>
<th>L-NMMA + L-arginine hydrochloride</th>
<th>Vasopressin</th>
<th>Vasopressin + L-arginine hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Heart rate (beats/min)</td>
<td>-88±8*</td>
<td>-38±5*†</td>
<td>-86±9*</td>
<td>-71±12*</td>
</tr>
<tr>
<td>Δ Mean blood pressure (mm Hg)</td>
<td>43±3*</td>
<td>23±3*†</td>
<td>34±2*</td>
<td>34±2*</td>
</tr>
<tr>
<td>Δ Renal conductance (%)</td>
<td>-38±4*</td>
<td>-19±3*†</td>
<td>-24±3*</td>
<td>-21±5*</td>
</tr>
<tr>
<td>Δ Mesenteric conductance (%)</td>
<td>-53±2*</td>
<td>-32±3*†</td>
<td>-57±2*</td>
<td>-56±2*</td>
</tr>
<tr>
<td>Δ Hindquarters conductance (%)</td>
<td>-51±3*</td>
<td>-39±5*†</td>
<td>-51±4*</td>
<td>-46±5*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. L-Arginine hydrochloride (300 mg/kg) was given 10 minutes after injection of N°-monomethyl-L-arginine (L-NMMA) or 10–15 minutes after onset of vasopressin infusion. Measurements were made 5 minutes after L-arginine hydrochloride administration. Δ, change from baseline; n, number of rats in the group.

* p<0.05 vs. baseline; † p<0.05 vs. L-NMMA alone (Friedman's test).
vascular conductance (Table 1). However, L-arginine hydrochloride (100 mg/kg) attenuated the vasoconstrictor effects of L-NMMA (Figure 8) causing transient rises in mesenteric and hindquarters vascular conductances that rapidly fell but to levels higher than those before its administration. Ten minutes after administration of L-arginine hydrochloride (100 mg/kg), the renal vascular conductance had returned to the basal level, but mesenteric and hindquarters vascular conductances were still reduced 18±6 and 32±5% below basal levels, respectively (p<0.05).

L-Arginine hydrochloride (300 mg/kg) significantly attenuated all the cardiovascular effects of L-NMMA, without affecting those of vasopressin (Table 2).

Discussion

Recent studies have shown that L-NMMA induces an elevation in blood pressure in the anesthetized rabbit and the guinea pig. This hypertensive response was associated with an inhibition of endothelium-dependent vasodilation induced by acetylcholine and was accompanied by inhibition of the release of NO from the aorta of the treated animals. These observations clearly implicate the formation of NO from L-arginine in the maintenance of blood pressure and pose the question as to the role of NO in the regulation of regional blood flow in conscious animals.

We have now shown that L-NMMA causes dose-dependent increases in mean blood pressure in conscious rats. These effects are associated with renal, mesenteric, hindquarters, and internal carotid vasoconstriction, which is similar in all vascular beds studied. All these effects are accompanied by bradycardia that, as shown in the rabbit and the guinea pig, is probably reflex in nature.

The hypertensive response to L-NMMA, as well as its regional vasoconstrictor actions, are rapid in onset and both are attenuated by L-arginine. Furthermore, L-arginine has no sustained influence on the cardiovascular actions of vasopressin. These findings are consistent with inhibition by L-NMMA of the production of NO by vascular endothelial cells, rather than an effect mediated by an action on the arginine: NO pathway in other cells.

The ability of L-arginine to reverse the vasoconstriction induced by L-NMMA varied between vascular beds; although the responses in the renal and mesenteric vasculatures, respectively, were totally or almost totally reversed, the conductances in the internal carotid and hindquarters were only partially restored to pretreatment levels. The reasons for these findings are not clear at present but, as all vascular beds respond similarly to L-NMMA after 5 minutes, it is unlikely that the differences in the responses to L-arginine are due to regional differences in the activity of the L-arginine: NO pathway, or in the sensitivity to the NO formed. Whether variation in the reflex control of the different vascular beds, in the uptake of L-arginine, or its ability to displace L-NMMA account for the differences observed remains to be established. The mechanisms underlying the differences observed between vascular beds in the first 5 minutes after administration of L-NMMA also require investigation.

Under basal conditions, L-arginine acetate, D-arginine acetate, and L-lysine acetate all caused transient mesenteric vasodilatations. These effects are probably due to the acetate moiety (a known vasodilator) as D-arginine and L-lysine are not substrates for the NO-forming enzyme, and mesenteric vasodilatation is not seen with L-arginine hydrochloride alone. Because none of these effects can be attributed to the conversion of L-arginine to NO, it is likely that substrate availability is not rate limiting under resting conditions, confirming previous observations in vitro and in vivo.

The present observations clearly indicate that the overall conductance of all the vascular beds studied is regulated by NO generated from L-arginine. The mechanisms that control the activation of this pathway remain to be established, but could involve mechanical stimulation of endothelial cells due to shear stress resulting from pulsatile flow or other physical factors. This would be consistent with recent observations in intact vascular networks in vitro.

This and our previous results show that in the vasculature there is a substantial and functionally significant NO-mediated vasodilator tone that may be the main determinant of blood flow and on which local or systemic vasoconstrictor influences act.

Acknowledgment

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References

10. Aisaka K, Gross SG, Griffith OW, Levi R: N6-Methylarginine, an inhibitor of endothelium-derived nitric oxide synthesis, is a


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