Original Contributions

Neural Mechanism of Hypertension by Nitric Oxide Synthase Inhibitor in Dogs

Noboru Toda, Yoshihiko Kitamura, and Tomio Okamura

This study aimed to determine the mechanism of hypertension associated with nitric oxide synthase inhibition. Intravenous injections of \(N^G\)-nitro-\(L\)-arginine, a nitric oxide synthase inhibitor, produced a sustained increase in systemic blood pressure and a decrease in heart rate in anesthetized dogs, whereas \(N^G\)-nitro-\(D\)-arginine had no effect. \(L\)-Arginine reversed the pressor response. \(N^G\)-Nitro-\(L\)-arginine–induced hypertension was markedly attenuated or abolished by treatment with hexamethonium; this inhibition was still observed when the blood pressure fall caused by the ganglionic blocking agent was compensated by continuous infusion of angiotensin II. In dogs treated with phentolamine in a dose sufficient to lower blood pressure to the level similar to that elicited by hexamethionium and to suppress the pressor response to norepinephrine, the hypertensive effect of \(N^G\)-nitro-\(L\)-arginine was not attenuated. We conclude that hypertension caused by the nitric oxide synthase inhibitor is associated with an elimination of nitrooxidergic neural function rather than an impairment of the basal release of nitric oxide from the endothelium. (Hypertension 1993;21:3–8)

KEY WORDS • \(N^G\)-nitro-\(L\)-arginine • blood pressure • hypertension, experimental • nitric oxide

Nitric oxide (NO) or \(S\)-nitrosothiol that intracellularly liberates NO is regarded as endothelium-derived relaxing factor,\(^1\)\(^2\) and its synthesis is inhibited by guanidino-substituted \(L\)-arginine analogues, such as \(N^G\)-monomethyl \(L\)-arginine, \(N^G\)-nitro-\(L\)-arginine (L-NA), and L-NA methyl ester.\(^4\)\(^6\) Intravenous injections of NO synthase inhibitors raise systemic blood pressure in anesthetized and conscious rats and anesthetized guinea pigs, rabbits, and dogs.\(^7\)\(^11\) and intra-arterial injections decrease blood flow and increase vascular resistance.\(^12\)\(^13\) The pressor and vasoconstrictor effects of NO synthase inhibitors are prevented by \(L\)-arginine but not by \(D\)-arginine. The hypertensive effects of NO synthase inhibitors are considered to be due to the suppression of the basal release of NO from the endothelium of resistance vessels. However, whether the NO inhibitor originates solely from the endothelium has not been fully determined.

NO transmits information from nonadrenergic, non-cholinergic vasodilator nerves to smooth muscle in dog and monkey cerebral arteries.\(^14\)\(^16\) In addition, the presence of NO synthase in perivascular nerve has been demonstrated histochemically.\(^17\) Therefore, NO is thought to act as a transmitter,\(^18\) and the nerve is called “nitrooxidergic.”\(^19\)\(^20\) We have also demonstrated the reciprocal innervation of adrenergic, vasoconstrictor and nitrooxidergic, vasodilator nerves in dog and monkey temporal and mesenteric arteries.\(^19\)\(^22\) Treatment with NO synthase inhibitors potentiates the vasoconstrictor response to adrenergic nerve stimulation, possibly because of the abolition of vasodilator nerve function.\(^19\)\(^22\)

In a recent study, we observed that intraluminally applied L-NA potentiates the neurally induced vasoconstriction, as does extraluminal L-NA (Zhang et al, unpublished data).

We undertook the present study to elucidate whether elimination of neurogenic vasodilator control is involved in the hypertension induced by L-NA administered to anesthetized dogs.

Methods

Thirty-two mongrel dogs of both sexes (9–16 kg) were used for study. All animals were fed standard chow and water ad libitum and were housed according to institutional guidelines at the Shiga University of Medical Sciences. These studies were approved by the Animal Rights Committee, Shiga University of Medical Sciences. Dogs were anesthetized with sodium pentobarbital (25 mg/kg i.p.) and were intubated. Supplemental doses of the anesthetic were applied via the femoral vein when necessary. The animals were permitted to breathe spontaneously. Arterial systolic and diastolic blood pressures were monitored with a pressure transducer (MPU0.5, TMI Inc., Tokyo) and an amplifier (type 1236, NEC Sanei, Tokyo) via a catheter inserted into the left femoral artery. Heart rate was monitored by a cardiotachometer. Drugs were injected into the right femoral vein. L-NA (10 mg/kg) was dissolved in saline (10 ml) and slowly injected for 2 minutes into the vein. Studies on L-NA were carried out once in each dog, unless the washout period of 1 week was used. The animals were treated with 4 mg/kg hexamethonium, and then the drug (0.4 mg/kg per minute) was continuously infused. After a reversal of the reflex decrease in heart rate by norepinephrine to tachycardia was determined,

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Received April 21, 1992; accepted in revised form September 21, 1992.
L-NA was administered. To compensate the depressor action of hexamethonium, angiotensin II (0.1 μg/kg per minute i.v.) was continuously infused during the experimental period. Some dogs were treated with phentolamine (1 mg/kg i.v.) before the administration of L-NA.

Results in the text, figures, and tables are mean±SEM. Statistical analyses were made using the Student's t test and Tukey's test after one-way analysis of variance. Drugs used were L-NA, N⁶-nitro-D-arginine, angiotensin II (all from Peptide Institute, Minoh, Japan), L-arginine (Nacalai Tesque, Kyoto, Japan), hexamethonium bromide (Yamanouchi Co., Tokyo), phen- tolamine mesylate (CIBA-GEIGY, Takarazuka, Japan), aspirin DL-lysine (Venopirin, Green Cross, Osaka, Japan), and dl-norepinephrine hydrochloride (Sankyo Co., Tokyo).

Results

Intravenous injections of L-NA (10 mg/kg) elicited a slowly developing hypertension in anesthetized dogs (Figure 1). The peak value was attained within 20–30 minutes and persisted for 60 minutes or longer. Mean values of the increment in systolic, mean, and diastolic blood pressures are shown in Figure 2 and Table 1. Heart rate decreased in association with the elevation of blood pressure. The induced hypertension and brady- cardia were reversed by injections of L-arginine (500 mg/kg); the mean increase in pressure by L-NA and the decrease by L-arginine were 20.4±2.0 and 17.6±1.4 mm Hg (n=7), respectively. N⁶-Nitro-D-arginine (10 mg/kg i.v.) did not alter systemic blood pressure (n=5; results not shown). The hypertensive effect of L-NA was not inhibited by treatment with aspirin (90 mg/kg i.v.) compared with the effect in nontreated dogs (23.3±2.1 mm Hg increase, n=12; Figure 2); the mean blood pressure increase averaged 25.4±3.7 mm Hg from the level of 102.8±5.6 mm Hg (n=5).

Treatment with hexamethonium in doses (4 mg/kg bolus injection plus 0.4 mg/kg per minute infusion) sufficient to reverse the reflex bradycardia by a hypertensive dose of norepinephrine (3 μg/kg) to tachycardia markedly suppressed or abolished the pressor effect of L-NA (Figure 3 and Table 2). Typical responses are illustrated in Figure 4. Treatment with phentolamine in a dose (1 mg/kg) sufficient to lower systemic blood pressure to the level almost identical to that caused by hexamethonium markedly depressed the pressor response to norepinephrine (Figure 5). Under these conditions, L-NA raised systolic, mean, and diastolic blood pressures (Figure 3 and Table 2) to a similar extent as those seen under control conditions (Figure 2 and Table 1). In four anesthetized dogs, the effectiveness of the blocking agents to diminish the hypertensive effect of L-NA was compared (Table 3). In dogs 1 and 2, the effect of hexamethonium was determined first, and 1 week later the experiment with phentolamine was carried out. In dogs 3 and 4, phentolamine was used first, and the study on hexamethonium was performed 1 week later. Even when the order of treatment with these agents was changed, hexamethonium was always effective in depressing the response to L-NA.

In addition, the effectiveness of hexamethonium was determined in the dogs in which systemic blood pressure was compensated by continuous, intravenous infusions.
TABLE 1.  Effects of N\(^{\text{O}}\)-Nitro-L-Arginine (10 mg/kg i.v.) on Systolic and Diastolic Blood Pressures and Heart Rate in Anesthetized Control Dogs and Those Treated With Hexamethonium and Angiotensin II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12</td>
<td>120.8±5.8</td>
<td>67.5±4.5</td>
<td>163.7±4.2</td>
</tr>
<tr>
<td>L-NA</td>
<td>12</td>
<td>143.2±6.1*</td>
<td>91.0±4.6†</td>
<td>139.6±2.7†</td>
</tr>
<tr>
<td>(\Delta) by L-NA</td>
<td>12</td>
<td>22.3±2.7</td>
<td>23.5±2.1</td>
<td>-24.1±3.2</td>
</tr>
<tr>
<td>Treated dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5</td>
<td>129.4±2.6</td>
<td>70.8±2.8</td>
<td>187.8±7.1</td>
</tr>
<tr>
<td>(C_6)</td>
<td>5</td>
<td>84.6±6.2†</td>
<td>45.4±4.4†</td>
<td>153.4±12.8*</td>
</tr>
<tr>
<td>(C_6)+Ang II</td>
<td>5</td>
<td>130.4±2.0‡</td>
<td>87.4±1.8*‡</td>
<td>141.0±9.2*</td>
</tr>
<tr>
<td>(C_6)+Ang II+L-NA</td>
<td>5</td>
<td>138.2±3.4‡</td>
<td>96.2±1.7†‡</td>
<td>142.2±8.9*</td>
</tr>
<tr>
<td>(\Delta) by L-NA</td>
<td>5</td>
<td>7.8±2.2§</td>
<td>7.6±0.7§</td>
<td>1.2±3.5</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; L-NA, \(N^{\text{O}}\)-nitro-L-arginine; \(\Delta\), changes in responses to L-NA compared with values before administration of L-NA; \(C_6\), hexamethonium; Ang II, angiotensin II. Values are mean±SEM. Data were obtained when responses to drugs were stabilized.

\(p<0.05; \, t<0.01\), compared with None.

\(p<0.01\), compared with \(C_6\) (Tukey's test).

\(§<0.05; \, ||p<0.01\), compared with \(\Delta\) by L-NA in control dogs (unpaired \(t\) test).

of angiotensin II (0.1 \(\mu\)g/kg per minute). Systolic, mean, and diastolic blood pressure values and heart rate are summarized in Figure 2 and Table 1. L-NA did not significantly raise pressures in the hexamethonium-treated dogs, which contrasted with results obtained from nontreated control dogs.

**Discussion**

Intravenous injections of L-NA, an NO synthase inhibitor, produced a slowly developing, persistent hypertension in anesthetized dogs, as in rats, guinea pigs, and rabbits,\(^7^\)\(^-^9\) whereas \(N^{\text{O}}\)-nitro-D-arginine did not alter systemic blood pressure. L-Arginine reversed the L-NA-induced pressor response. These findings suggest that the induced hypertension is associated with a suppression of the synthesis and release of NO. Klubunde et al\(^11\) have reported that the pressor response of anesthetized dogs to a NO synthase inhibitor, \(N^{\text{O}}\) monomethyl L-arginine, is abolished by treatment with indomethacin. However, in the present study, treatment with aspirin in a dose sufficient to abolish the release of prostaglandins\(^23\) did not significantly alter the hypertensive effect of L-NA, suggesting that the involvement of cyclooxygenase products is excluded.

Hypertensive effects of NO synthase inhibitors have been postulated to derive from a suppression of basal release of NO from the endothelium. However, we proposed the possibility that the hypertension is caused by impairment of neurogenic vasodilatation that would be mediated by NO.\(^19\)\(^-^22\) The reasons are 1) potentiation of the pressor response to adrenergic nerve stimulation in isolated, perfused dog mesenteric artery segments is elicited by NO synthase inhibitors applied intraluminally (Zhang et al, unpublished data) as well as extraluminally\(^14\)\(^-^19\); 2) this potentiation is not associated with an increase in the response to exogenous norepinephrine and in the release of the amine from stimulated adrenergic nerves\(^19\)\(^-^21\); and 3) relaxations caused by NO synthase inhibitors were not seen in the isolated segments.

![ANESTHETIZED DOGS](image_url)
by transmural electrical stimulation of mesenteric and temporal arteries treated with α-adrenergic receptor blockers are abolished by NO synthase inhibitors; involvement of the nitroxidergic nerve in the relaxation is hypothesized. To determine the neural mechanism of hypertension, we tested the effect of hexamethonium, a ganglionic blocking agent, on the pressor response to L-NA. Hexamethonium lowered blood pressure in a dose sufficient to reverse the reflex decrease in heart rate caused by norepinephrine to an increase (Figure 4) and almost abolished the pressor response to L-NA. The ability of this blocking agent to abolish the L-NA action cannot be considered to result from a fall of blood pressure, because the hypertension associated

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>HR (bpm)</th>
</tr>
</thead>
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<tr>
<td><strong>Phentolamine series</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5</td>
<td>130.6±9.6</td>
<td>73.0±5.8</td>
<td>171.0±7.3</td>
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<tr>
<td>Phentol</td>
<td>5</td>
<td>87.2±7.0*</td>
<td>51.2±6.4†</td>
<td>199.2±23.1</td>
</tr>
<tr>
<td>Phentol+L-NA</td>
<td>5</td>
<td>117.4±8.5‡</td>
<td>76.4±5.8§</td>
<td>182.8±18.9</td>
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<tr>
<td>Δ by L-NA</td>
<td>5</td>
<td>28.2±4.6</td>
<td>25.2±6.8</td>
<td>−16.4±6.9</td>
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<tr>
<td><strong>Hexamethonium series</strong></td>
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<td></td>
<td></td>
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<tr>
<td>None</td>
<td>6</td>
<td>136.2±7.3</td>
<td>76.8±5.1</td>
<td>194.7±7.0</td>
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<tr>
<td>C6</td>
<td>6</td>
<td>89.2±7.5*</td>
<td>53.2±5.8†</td>
<td>132.5±5.3*</td>
</tr>
<tr>
<td>C6+L-NA</td>
<td>6</td>
<td>93.2±7.8*</td>
<td>58.2±7.3†</td>
<td>132.4±10.0*</td>
</tr>
<tr>
<td>Δ by L-NA</td>
<td>6</td>
<td>4.0±±2.0‡</td>
<td>5.0±2.8§</td>
<td>−4.4±2.9</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; Phentol, phentolamine; L-NA, N⁶-nitro-L-arginine; Δ, changes in responses to L-NA compared with values before administration of L-NA; C₆, hexamethonium. Values are mean±SEM. Data were obtained when responses to drugs were stabilized.

 devastation by L-NA in phentolamine-treated dogs (unpaired t test).

**FIGURE 4.** Tracings show effects of N⁶-nitro-L-arginine (L-NA) (10 mg/kg i.v.) on systemic blood pressure (top) and heart rate (bottom) in anesthetized dog treated with hexamethonium (C₆). NE, 3 μg/kg norepinephrine.

**FIGURE 5.** Tracings show effects of N⁶-nitro-L-arginine (L-NA) (10 mg/kg i.v.) on systemic blood pressure (top) and heart rate (bottom) in anesthetized dog treated with phenolamine. NE, 3 μg/kg norepinephrine.
with L-NA was not reduced in dogs treated with phen tolamine in a dose that lowered blood pressure similarly to hexamethonium. In addition, the effect of L-NA was not restored even when blood pressure was raised by continuous infusion of angiotensin II to a level almost identical to that before the administration of hexa methonium. Contrasting effects of hexamethonium and phen tolamine were seen in the same dogs to which both drugs were applied at an interval of 1 week (Table 3). Similar results with another ganglionic blocking agent, chlorisondamine, were also obtained in anesthetized rats, in which hypertension and renal vasoconstriction induced by L-NA were abolished by treatment with the blocking agent. In this study, the hypertensive effect was not influenced in the rats in which blood pressure was lowered by hydralazine. These findings indicate that neurogenic factors play a major role in the pressor and vas constrictor responses to NO synthase inhibitors. The induced hypertension would be associated with an impairment of NO production in nitroxidergic nerves rather than the endothelium.

Pegoraro et al have demonstrated that hypertension induced by N°-monomethyl L-arginine and L-NA methyl ester is not influenced by treatment with hexamethonium and pithing in anesthetized rats. The reason for the discrepancy between their results and those of ourselves and Lacolley et al cannot be explained. However, ineffectiveness of pithing in the response to NO synthase inhibitors does not necessarily rule out the possible involvement of nitroxidergic neural influence, because NO synthase and parasympathetic transmitter are demonstrated to coexist in ganglia.

Recent study has demonstrated that intravenous injections of N°-monomethyl L-arginine increase sympathetic renal nerve activity in anesthetized rats, in association with an increase in blood pressure, and suggested the central regulation by NO of sympathetic tone. In the present study, however, hypertensive effects of L-NA were not influenced in dogs treated with phen tolamine in a dose in which the pressor response to norepinephrine was markedly inhibited. Phen tolamine causes a reflex increase in sympathetic efferent nerve activity and a blockade of α-adrenergic receptors in smooth muscle, possibly minimizing the action of centrally activated sympathetic tone. In addition, L-NA injected into the subarachnoid space via the cisterna magna does not raise systemic blood pressure in anesthetized rats (Toda et al, unpublished data). These results suggest that the central action is, if any, minimal in anesthetized dogs under the experimental conditions used. Therefore, we conclude that NO synthase inhibitors impair the function of nitroxidergic, vasodilator nerves innervating the arterial wall because of a suppression of synthesis of transmitter NO.

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Neural mechanism of hypertension by nitric oxide synthase inhibitor in dogs.
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Hypertension. 1993;21:3-8
doi: 10.1161/01.HYP.21.1.3

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