Mechanism of Neurally Induced Monkey Mesenteric Artery Relaxation and Contraction

Noboru Toda and Tomio Okamura

Physiological importance in vasodilator innervation alleviating noradrenergic neurogenic vasoconstriction has not been clarified. Isolated monkey mesenteric artery strips denuded of the endothelium responded to nerve stimulation by electrical pulses or nicotine with a contraction, which was potentiated by \( N^G \)-nitro-L-arginine, a nitric oxide synthesis inhibitor, but not by the D-enantiomer. The potentiation was abolished by L-arginine. \( N^G \)-Nitro-L-arginine did not potentiate the response to exogenous norepinephrine nor did it increase the release of \(^3\)H\)norepinephrine from adrenergic nerves electrically stimulated. The contraction was reversed by treatment with phentolamine and guanethidine to a relaxation, which was abolished by \( N^G \)-nitro-L-arginine. The inhibition was reversed by L- but not D-arginine. The relaxant response was not influenced by atropine, timolol, or indomethacin. These findings strongly suggest the importance of reciprocal nitric oxide-related (nitroxidergic) vasodilator and noradrenergic vasoconstrictor innervation in the regulation of monkey arterial tone. (Hypertension 1992;19:161-166)

Noradrenergic innervation in the vascular wall plays a crucial role in the physiological control of vasomotor tone, thus regulating peripheral vascular resistance and blood distribution in organs and tissues. The vasoconstrictor innervation also participates in pathogenesis of vascular disorders such as vasospasm and hypertension. Despite a histochemical demonstration of cholinergic nervelike structures, the functional roles of these structures have not been clarified. Transmitter substances from noradrenergic and cholinergic nerves seem to act synergistically on smooth muscle since cholinergic nerve stimulation constricts bovine coronary arteries and canine veins. Nonadrenergic, noncholinergic vasodilator innervation has been demonstrated in human, monkey, dog, cat, and bovine cerebral arteries. Recent studies in our laboratory dealing with monkey and dog cerebral arteries have provided evidence that nitric oxide (NO) plays a crucial role in transmitting information from the nerve to arterial smooth muscle. Similar results were also obtained in rat anococcygeus muscle and dog ileocolonic junction. In the present study, we demonstrated the presence of nonadrenergic, noncholinergic vasodilator innervation in monkey mesenteric arteries, determined the vasodilator and vasoconstrictor nerve interaction, and clarified the involvement of NO in neurally induced vasodilatation by the use of \( N^G \)-nitro-L-arginine (L-NA), an NO synthesis inhibitor.

Methods

Japanese monkeys (Macaca fuscata, 4 years or older) of either sex, weighing 6-10 kg, were anesthetized with intramuscular injections of ketamine (40 mg/kg) and were killed by exsanguination from the carotid arteries. The age of wild monkeys was determined up to 7 years by the growth and the eruption of teeth. The superior mesenteric arteries of 0.4-0.6 mm o.d. were isolated. Helically cut strips of the arteries, denuded of the endothelium, were vertically fixed between hooks under a resting tension of 1 g in a muscle bath (20 ml capacity) containing a modified Ringer-Locke solution that was aerated with 95% \( O_2 \)-5% \( CO_2 \) and was maintained at 37°C. Endothelium denudation was determined by abolishment of acetylcholine-induced relaxations. Constituents of the solution were as follows (mM): NaCl 120, KCl 5.4, CaCl\(_2\) 2.2, MgCl\(_2\) 1.0, NaHCO\(_3\) 25.0, and dextrose 5.6. The pH of the solution was 7.35-7.42. Before the start of experiments, all of the strips were allowed to equilibrate in the bathing media for 60-90 minutes, during which time the fluids were replaced every 10-15 minutes.

All of the arterial strips, except for those used for experiments with nicotine and NO, were placed between stimulating electrodes. The gap between the strip and the electrodes was wide enough to allow
The content of cyclic GMP in monkey mesenteric arteries was measured by a reported method. 

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The results shown in the text, figures, and tables are expressed as mean±SEM. Statistical analyses were made using Student's paired and unpaired t test and Tukey's method after one-way analysis of variance. Drugs used were N^6^-nitro-L-arginine (L-NA), N^6^-nitro-D-arginine (D-NA) (Peptide Institute, Minoh, Japan), L-arginine and D-arginine, nicotine (Nacalai Tesque, Kyoto, Japan), tetrodotoxin, 6-norepinephrine hydrochloride (Sankyo, Tokyo), PGF^2, (Ono, Osaka), indomethacin, guanethidine sulfate (Sigma Chemical Co., St. Louis, Mo.), atropine sulfate (Tanabe, Tokyo), timolol maleate (Banyu, Tokyo), phenotiamine mesylate (CIWA-GEIGY, Japan, Takarazuka, Japan), hexamethonium bromide (Yamanouchi, Tokyo), and papaverine hydrochloride (Daippon, Osaka). Responses to NO were obtained by adding the NaNO_3 solution adjusted at pH 2. Concentrations of acidified NaNO_3 in the muscle bath were presented as those of NO. Oxyhemoglobin (oxyHb) was prepared by addition of 10-fold molar excess of the reducing agent, sodium dithionite, to a 1 mM solution of commercial Hb (Sigma) in distilled water. Sodium dithionite was then removed by extensive dialysis.

Results

Effects of Transmural Electrical Stimulation on Mesenteric Arteries

Transmural electrical stimulation (2, 5, and 20 Hz) produced a frequency-related contraction in the monkey mesenteric artery strips denuded of the endothelium. Contractions induced by stimulation were significantly different from control and the value with L-NA+L-arg (p<0.01). Number of strips from separate monkeys: 10 for electrical stimulation and L-NA, five for electrical stimulation and D-NA, and 11 for nicotine. Vertical bars represent SEM.
endothelium, under resting conditions and when partially contracted with PGF2α (49±21, 135±20, and 238±39 mg, n=5, respectively). The contraction was markedly suppressed or abolished by treatment with 10⁻⁷ M phentolamine (n=18), 10⁻⁶ M guanethidine (n=5), or 3×10⁻⁷ M tetrodotoxin (all the strips used). The stimulation-induced contraction was potentiated by treatment with 10⁻⁶ M L-NA, as shown in Figure 1. L-NA did not contract the arteries under resting conditions. The potentiating effect was not influenced by 3×10⁻⁴ M D-arginine (n=4) but was antagonized by treatment with 3×10⁻⁴ M L-arginine (Figure 1). The typical result is illustrated in Figure 2, upper tracing. D-NA (10⁻⁶ M) did not alter the response (Figure 1). Contractions of the artery strips in response to exogenously applied norepinephrine (2×10⁻⁸, 10⁻⁷, and 5×10⁻⁷ M) were not significantly increased by 10⁻⁶ and 10⁻⁵ M L-NA (Figure 3). In the strips from the same monkeys, contractions induced by 5 Hz stimulation averaged 93±26 mg (n=7) under resting conditions.

The [³H] overflow evoked by transmural electrical stimulation (5 Hz for 3 minutes) from superfused monkey mesenteric artery strips previously incubated in the bathing media containing [³H]norepinephrine was not increased by treatment with 5×10⁻⁶ M L-NA. The overflow ratio S6/S3 was 1.00±0.06 in five control series of experiments and 0.87±0.02 in five L-NA-treated series.

In the arteries treated with 10⁻⁷ M phentolamine (Figure 2) or 10⁻⁶ M guanethidine (Figure 4) and partially contracted with PGF2α, transmural electrical stimulation produced a relaxation or a slight contraction followed by a relaxation; relaxations by the stimulation at 2, 5, and 20 Hz averaged 6.1±2.2% (n=4), 14.4±1.8% (n=24), and 20.3±4.7% (n=4), respectively. Treatment with 10⁻⁶ M L-NA markedly suppressed the relaxation at 5 Hz stimulation (Figure 4) or reversed it to a contraction (Figure 2, lower tracing). The quantitative data are summarized in Figure 5. The inhibitory effect was antagonized by L-arginine (3×10⁻⁴ M) (Figure 5) but not by D-arginine (from 3.0±1.2% contraction relative to that caused by 30 mM K⁺ to 3.1±1.2%, n=4). The stimulation (5 Hz)-induced relaxations were abolished by 3×10⁻⁷ M tetrodotoxin (all of the preparations used) and 1.6×10⁻³ M oxyHb (n=5) but were unaffected by treatment with 10⁻⁷ M timolol, 10⁻⁷ M atropine, and 10⁻⁴ M indomethacin (Table 1). L-Arginine (3×10⁻⁴ M) did not relax the control and L-NA-treated strips. NO (10⁻⁷ M)-induced relaxations were not significantly influenced by 10⁻⁶ M L-NA (59.0±4.5 versus 51.9±5.2%, n=10).

Effects of Nicotine on Mesenteric Arteries

The addition of nicotine (10⁻⁴ M) produced a phasic contraction in the monkey mesenteric arteries denuded of the endothelium, which was potentiated by treatment with 10⁻⁶ M L-NA (Figure 1). The

![Figure 2](image-url)  
**Figure 2.** Representative tracings show responses to transmural electrical stimulation (5 Hz) of a monkey mesenteric artery strip without endothelium, before and after treatment with N⁰-nitro-L-arginine (L-NA) (10⁻⁶ M), D-arginine (D-arg.) and L-arginine (L-arg.) (3×10⁻⁴ M), and tetrodotoxin (TTX) (3×10⁻⁷ M) in control (upper tracing) and phenolamine-containing media (lower tracing). After the experiment sequence on the upper tracing was over, the strip was repeatedly washed and equilibrated. Then the strip was partially contracted with 3×10⁻⁴ M prostaglandin F₂α (from the level shown as a horizontal line) and treated with 10⁻⁷ M phenolamine. PA, 10⁻⁴ M papaverine to attain the maximal relaxation. X, application of electrical stimulation under TTX-treated conditions.

![Figure 3](image-url)  
**Figure 3.** Line graph shows concentration–contractile response curves for norepinephrine before and after treatment with N⁰-nitro-L-arginine (L-NA) (10⁻⁶ and 10⁻⁵ M) in monkey mesenteric artery strips denuded of endothelium. Numbers in parentheses indicate the number of strips from separate monkeys. Vertical bars represent SEM.
potentiation was reversed by $3 \times 10^{-4}$ M L-arginine. The arteries treated with $10^{-6}$ M phentolamine or $10^{-5}$ M guanethidine and contracted with PGF$_{2\alpha}$ responded to nicotine with a relaxation, which was abolished or converted to a slight contraction by $10^{-6}$ M L-NA (Figure 6). The inhibition was reversed by $3 \times 10^{-4}$ M L-arginine but not by its D-enantiomer ($n=4$). Typical responses are shown in Figure 7. The nicotine-induced relaxation was not influenced by $10^{-7}$ M timolol and $10^{-7}$ M atropine (Table 1) but were abolished by $10^{-5}$ M hexamethonium ($n=6$).

The level of cyclic GMP in monkey mesenteric artery strips without the endothelium was measured. Nicotine ($10^{-4}$ M) significantly increased cyclic GMP in the strips, and treatment with $10^{-5}$ M hexamethonium abolished the stimulating effect of nicotine. The results are summarized in Table 2.

**Discussion**

Vasoconstrictor responses to noradrenergic nerve stimulation by electrical pulses or nicotine were potentiated by L-NA in monkey mesenteric arteries (present study) and by $N^\text{G}$-monomethyl-L-arginine, the other NO synthesis inhibitor, in dog mesenteric arteries but not by the D-enantiomers. The potentiation was abolished by the addition of L-arginine but not D-arginine. The $[\text{H}]$ overflow evoked by adrenergic nerve stimulation from artery strips previously incubated in $[\text{H}]$norepinephrine was not increased by L-NA nor was the contractile response to exogenously applied norepinephrine. Therefore, the induced potentiation of the neurally induced contraction would be due to a suppression of vasodilatation possibly mediated by NO rather than an increase in the stimulation-induced release of norepinephrine and the sensitivity of arteries to the amine. Treatment with bretylium or guanethidine, adrenergic neuron blockers, and 6-hydroxydopamine (N. Toda and T. Okamura, unpublished data) abolished the contractile response to adrenergic nerve stimulation but did not inhibit the relaxation elicited by the dilator nerve stimulation, suggesting that norepinephrine and the vasodilator transmitter do not coexist in the same nerve.

Relaxant responses to transmural electrical stimulation and nicotine in monkey mesenteric artery strips treated with phentolamine or guanethidine were not influenced by timolol and atropine but were abolished by tetrodotoxin (for electrical stimulation) or hexamethonium (for nicotine). The responses were abolished or converted to contractions by treatment with L-NA, the inhibitory effect being antagonized by L-arginine but not D-arginine. Similar results were also obtained in monkey and dog cerebral arteries that responded to the nerve stimulation only with relaxations even in the absence of blockade of adrenergic nerves and $\alpha$-adrenergic receptors. The relaxations were abolished by hemoglobin and methylene blue. In the present study, nicotine increased the level of cyclic GMP in the monkey mesenteric arteries without the endothelium; the effect was abolished by hexamethonium. It may therefore be concluded that the neurally induced relaxation is associated with NO released from nonadrenergic, noncholinergic nerves that activate guanylate cyclase and increase the synthesis of cyclic GMP.

**TABLE 1. Effects of Inhibitors on Relaxant Response to Transmural Electrical Stimulation and Nicotine of Monkey Mesenteric Artery Strips Denuded of Endothelium and Treated With Phentolamine**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Relaxation (%)$^*$ induced by TES (5 Hz)</th>
<th>Nicotine ($10^{-4}$ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>D-NA ($10^{-6}$ M)</td>
<td>6</td>
<td>16.5±3.2</td>
</tr>
<tr>
<td>Atropine ($10^{-7}$ M)</td>
<td>5</td>
<td>17.8±4.9</td>
</tr>
<tr>
<td>Timolol ($10^{-7}$ M)</td>
<td>5</td>
<td>18.2±4.7</td>
</tr>
<tr>
<td>IM ($10^{-4}$ M)</td>
<td>4</td>
<td>11.5±3.9</td>
</tr>
</tbody>
</table>

TES, transmural electrical stimulation; $n$, number of strips from separate monkeys; D-NA, $N^\text{G}$-nitro-D-arginine; IM, indomethacin.

$^*$Relaxations relative to those induced by $10^{-4}$ M papaverine.
immunohistochemical study by Bredt et al.\textsuperscript{20} indicates that NO synthase–containing nerves are located in the adventitia of rat large cerebral arteries but not in the media. They provided supportive evidence for our hypothesis that NO liberated from the nerves (putatively nitroxidergic) transmits vasodilator information to smooth muscle cells.

The role of cholinergic innervation in vasodilatation has not been clarified. However, our data obtained so far strongly suggest that vasodilator nitroxidergic and vasoconstrictor noradrenergic nerves participate importantly in the regulation of vascular tone by balancing dilatation and constriction. In most vasculatures, neuronal vasoconstriction predominates over the vasodilatation. In the present and previous (Reference 11 and N. Toda and T. Okamura, unpublished data) studies, L-NA markedly suppressed the vasodilator nerve function in monkey mesenteric, superficial temporal, and cerebral arter-

\textbf{TABLE 2. Cyclic GMP Levels of Monkey Mesenteric Artery Strips Denuded of Endothelium and Exposed to Control Media and Those Containing Nicotine or Nicotine Plus Hexamethonium}

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cyclic GMP (fmol/mg wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.62±0.48</td>
</tr>
<tr>
<td>Nicotine (10^-4 M)</td>
<td>5.32±0.53</td>
</tr>
<tr>
<td>Nicotine+C6 (10^-5 M)</td>
<td>2.72±0.34</td>
</tr>
</tbody>
</table>

- \( n \), Number of artery strips from separate monkeys; C6, hexamethonium.
- *Frozen tissue weight.
- \(^{\dagger}\)p<0.01 significantly different from control.
- \(^{\dagger\dagger}\)p<0.01 significantly different (Tukey’s method) from value with nicotine plus hexamethonium.
ies, rendering noradrenergic nerve function more predominant in the former two arteries. We used mesenteric and temporal arteries as representatives of visceral and skeletal muscle/cutaneous arteries, respectively. Studies are underway to determine whether the other visceral, skeletal muscle, and cutaneous arteries are also innervated by nonadrenergic, noncholinergic vasodilator nerves. Systemic blood pressure in anesthetized rabbits was significantly raised by intravenous injections of L-NA, and the effect was reversed by L-arginine (T. Noda and T. Okamura, unpublished data). In anesthetized rabbits and guinea pigs and conscious rats, NO synthesis inhibitors also raised vascular resistance and systemic blood pressure. In addition, the inhibitor infused into the brachial artery decreases forearm blood flow in healthy volunteers. These authors postulated that increased vascular resistance induced by the synthesis inhibitors is due to a suppression of basal release of endothelium-derived NO that dilates arterioles.

In infarcted dog, guinea pig, and monkey cerebral arteries, L-NA of the nitroxidergic vasodilator nerve function in bovine basilar arteries. Am J Physiol 1990;258:H1511–H1517


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References


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