Neuronal Nitric-Oxide Synthase Inhibition Facilitates Adrenergic Neurotransmission in Rat Mesenteric Resistance Arteries

Yukako Hatanaka, Narumi Hobara, Jin Honghua, Shinji Akiyama, Hideki Nawa, Yuta Kobayashi, Fusako Takayama, Yutaka Gomita, and Hiromu Kawasaki

Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Tsushima-naka, Okayama, Japan (Y.H., N.H., J.H., S.A., F.T., H.K.); Department of Hospital Pharmacy, Okayama University Hospital of Medicine and Dentistry, Shikata-cho, Okayama, Japan (H.N., Y.G.); and Centre for Integrated Research in Science, Shimane University, Enya-cho, Izumo, Japan (Y.K.)

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ABSTRACT

The effects of nonselective nitric-oxide synthase (NOS) inhibitors [N-ω-nitro-L-arginine methyl ester (L-NAME) and N-ω-nitro-L-arginine (L-NNA)] and specific neuronal NOS (nNOS) inhibitor [vinyl-L-N-5-(1-imino-3-butenyl)-L-ornithine (L-VNIO)] on adrenergic nerve-mediated vasoconstriction were studied in rat perfused mesenteric vascular beds without endothelium. Perfusion of L-NAME, L-NNA, or L-VNIO markedly augmented vasoconstrictor responses to periarterial nerve stimulation (PNS; 2–8 Hz) without affecting vasoconstriction induced by exogenously injected norepinephrine (NE). Addition of L-arginine, a precursor for the synthesis of nitric oxide (NO), reversed the augmentation of the PNS response by L-NAME. The PNS (8 Hz)-evoked NE release in the perfusate was increased by L-NAME perfusion. In preparations treated with capsaicin [a depleter of calcitonin gene-related peptide (CGRP)-containing nerves], L-NAME did not augment vasoconstrictor responses to PNS or NE injection. Combined perfusion of CGRP(8-37) (a CGRP receptor antagonist) and L-NAME induced additive augmentation of the vasoconstrictor response to PNS but did not affect the response to NE injection. In preparations with active tone produced by methoxamine and in the presence of guanethidine, L-NAME perfusion did not affect the vasodilator response induced by PNS. Immunostaining of the mesenteric artery showed the presence of nNOS-like immunopositive nerve fibers, which were absent in arteries pretreated with capsaicin. These findings suggest that NO, which is released from perivascular capsaicin-sensitive nerves, presynaptically inhibits neurogenic NE release to modulate adrenergic neurotransmission.

When vascular adrenergic nerves are stimulated, peripheral vascular tone is increased mainly by released norepinephrine (NE) and partially by coreleased neuropeptide Y and ATP (Lundberg et al., 1982). Therefore, it is widely accepted that vascular adrenergic nerves mainly regulate the tone of the peripheral blood vessels. However, accumulating evidence has demonstrated that many blood vessels have innervation of nonadrenergic noncholinergic (NANC) nerves (Bevan and Brayden, 1987; Kawasaki et al., 1988; Toda and Okamura, 1992; Lee et al., 1996). Previous reports provided evidence that periarterial nerve stimulation (PNS) in rat mesenteric resistance arteries produces NANC neurogenic vasodilation (Kawasaki et al., 1988), which is mediated by calcitonin gene-related peptide (CGRP), a potent vasodilator neurotransmitter (Kawasaki et al., 1988). Our previous reports suggested that CGRPergic nerves suppress sympathetic nerve-mediated vasoconstriction via CGRP release; conversely, sympathetic nerves presynaptically inhibit the release of CGRP from the nerves to decrease CGRPergic nerve function (Kawasaki et al., 1990, 1991). Thus, we have proposed that CGRPergic vasodilator nerves along with sympathetic vasoconstrictor nerves regulate the tone of the mesenteric resistance artery. CGRPergic nerves are sensitive to capsaicin, a vanilloid receptor agonist that induces the release of CGRP from primary sensory neurons (Fujimori et al., 1989), finally leading to depletion of CGRP from the nerves.

Nitric oxide (NO) is synthesized from L-arginine by NO synthase (NOS) in various cells. NOS is present not only in

ABBREVIATIONS: NE, norepinephrine; NANC, nonadrenergic noncholinergic; PNS, periarterial nerve stimulation; CGRP, calcitonin gene-related peptide; NO, nitric oxide; NOS, nitric-oxide synthase; nNOS, neuronal NOS; L-NAME, N-ω-nitro-L-arginine methyl ester; L-NNA, N-ω-nitro-L-arginine; L-VNIO, vinyl-L-N-5-(1-imino-3-butenyl)-L-ornithine; SD, sodium deoxycholate; ACh, acetylcholine; LI, like immunoreactivity.
vascular endothelial cells (endothelial NOS) but also in perivascular nerves [neuronal NOS (nNOS)] (Moncada and Higgs, 1995; Sosunov et al., 1995). Inhibition of NOS by NOS inhibitors such as the nonselective NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) has been reported to augment the vasoconstrictor effect induced by PNS in various isolated blood vessels, whereas the enhanced PNS-induced responses to NOS inhibitors are reversed by L-arginine, a precursor for the synthesis of NO, and involve the endothelium-derived NO (Toda and Okamura, 1990; Postorino et al., 1998). On the other hand, several studies showed that NOS inhibitors have no effect on vasoconstriction in response to PNS in dog pulmonary arteries and human omental arteries without endothelium (Aldasoro et al., 1993; Segarra et al., 1998). In rat mesenteric arteries with endothelium, some reports have described that NOS inhibitors enhanced the vasoconstrictor responses to PNS (Yamamoto et al., 1997; Boric et al., 1999), suggesting the involvement of endothelial NO. However, it remains unclear whether perivascular NOS-containing nerves modulate adrenergic neurotransmission.

The aim of this study was to clarify the mechanism underlying the augmentation of adrenergic nerve-mediated vasoconstriction induced by nonselective NOS inhibitors, L-NAME and Nω-nitro-L-arginine (L-NNA), and a selective nNOS inhibitor, vinyl-nNOS (vinyl-1-N-(5-(1-imino-3-butyl)en)-L-ornithine (L-VNIO), in rat perfused mesenteric vascular beds. Additionally, the present study was designed to investigate the possible involvement of perivascular NANC nerves in the effects of NOS inhibitors. To avoid the potential confounding effects of endothelium-dependent NO release, the present study was performed in mesenteric arteries de-endothelialized with sodium deoxycholate (SD).

Materials and Methods

Perfusion of Mesenteric Vascular Beds and Perfusion Pressure Measurement. Male Wistar rats weighing 230 to 330 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the mesenteric vascular bed was isolated and prepared for perfusion as described previously (Kawasaki et al., 1988, 1990).

The isolated mesenteric vascular bed was perfused with Krebs' solution at a constant flow rate of 5 ml/min with a peristaltic pump (model AC-2120; Atto Bioscience, Rockville, MD) and superfused with the same solution at a rate of 0.5 ml/min to prevent drying. The Krebs' solution was bubbled with a mixture of 95% O₂ and 5% CO₂.

The modified Krebs' solution had the following composition: 119.0 mM NaCl, 4.7 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 1.2 mM KH₂PO₄, 0.03 mM EDTA-2Na, and 11.1 mM glucose, pH 7.4. Changes in the pressure measurement were measured with a pressure transducer (model TP-400T; Nihon Bunko, Tokyo, Japan) and recorded using a pen recorder (model U-228; Nippon Kayaku, Tokyo, Japan).

Chemical Removal of Vascular Endothelium. To remove the vascular endothelium, preparations with resting tone were perfused with 1.80 mg/ml SD in saline for 30 s as described previously (Takemura and Kawasaki, 1999; Shiraki et al., 2000). The preparations were then rinsed with SD-free Krebs' solution for 1 h.

PNS and Bolus Injection of NE. PNS at 2, 4, and 8 Hz was applied at 7-min intervals using bipolar platinum ring electrodes placed around the superior mesenteric artery. Rectangular pulses of 1 ms and supramaximal voltage (50 V) were applied for 30 s using an electronic stimulator (model SEN 3301; Nihon Bunko).

NE was directly injected into the perfusate proximal to the arterial cannula with an infusion pump (model 975; Harvard Apparatus Inc., Holliston, MA). A volume of 100 μl was injected during a period of 12 s.

Experimental Protocols for Preparations with Resting Tone. After responses to the first PNS (S₁) and NE injection (I₁) were obtained as the controls, the Krebs' solution was switched to Krebs' solution containing L-NAME (1, 10, or 100 μM), L-NNA (1, 10, or 100 μM), L-VNIO (10 or 100 μM), CGRP(8-37) (CGRP receptor antagonist, 100 nM), L-NAME (100 μM) plus L-arginine (10 or 100 μM or 1 mM), or L-NNAME (100 μM) plus CGRP(8-37) (100 nM), and then the second PNS (S₂) and NE injection (I₂) were carried out. Perfusion of these agents was begun 20 min before and continued throughout PNS or NE injection. To estimate the effects of the agents tested, changes in perfusion pressure in response to PNS or NE injection were expressed as the ratio between the vasocostriction induced by S₂ and S₁ or I₂ and I₁, respectively.

For capsaicin treatment, the preparation was perfused first with Krebs' solution containing 5 μM capsaicin for 20 min. After discontinuation of the capsaicin perfusion, the preparation was rinsed with capsaicin-free Krebs' solution for 30 min and subsequently perfused with sodium deoxycholate as described above.

At the end of each experiment, the preparations were perfused with Krebs' solution containing 2 μM methoxamine, a selective α₁ adrenoceptor agonist, to produce vascular tone and 5 μM guanethidine to block adrenergic neurotransmission. Chemical removal of the endothelium was assessed by verifying the lack of a relaxant effect after a bolus injection of 1 nmol acetylcholine (ACh). Thereafter, to assess the denervation of NANC nerves by capsaicin, 2 Hz PNS was applied.

Experimental Protocols for Preparations with Active Tone. The effect of L-NAME on CGRPergic nerve-mediated and exogenous CGRP-induced vasodilation was examined in denuded mesenteric vascular beds with active tone. After responses to the first PNS (S₁, 1 and 2 Hz) and CGRP (I₁, 10 pmol) injection were obtained as the control, the Krebs' solution containing 2 μM methoxamine and 5 μM guanethidine was switched to Krebs' solution containing 2 μM methoxamine, 5 μM guanethidine, and 100 μM L-NAME, and then the second PNS (S₂) and CGRP injection (I₂) were carried out. To quantify the effects of the peptides tested, changes in perfusion pressure in response to PNS or CGRP injection were expressed as the ratio between the vasodilation induced by S₂ and S₁ or I₂ and I₁, respectively.

At the end of each experiment, 100 μM papaverine was perfused to produce complete relaxation. Vasodilation was expressed as the percentage of the perfusion pressure at maximum relaxation induced by papaverine.

Measurement of NE in the Perfusate. In denuded preparations with resting tone, the perfusate was collected before and after the first PNS (S₁, 8 Hz) for 3 min. Thereafter, the Krebs' solution was switched to Krebs' solution containing L-NAME (100 μM), and then the perfusate was collected before and after the second PNS (S₂).

NE in the perfusate was adsorbed onto the alumina, and the extract obtained with acetic acid was assayed by high-performance liquid chromatography with an electrochemical detector (model HTEC-500; Eicom, Kyoto, Japan). The internal standard was 3,4-dihydroxybenzylamine hydrobromide (Sigma-Aldrich, St. Louis, MO).

Immunohistochemical Study. The mesenteric artery was isolated, fixed and immersed as described previously (Hobara et al., 2004). In another series of experiments, the isolated mesenteric artery was incubated with Krebs' solution containing capsaicin (500 μM) for 30 min and then rinsed with capsaicin-free Krebs' solution for 60 min. After rinsing, the mesenteric artery was fixed, and immunostaining was carried out as follows. The arteries were incubated with the primary antibody, anti-nNOS (1:500; raised in rabbit) (Zymed Laboratories, South San Francisco, CA), for 72 h at 4°C. After incubation, the site of the antigen-antibody reaction was revealed by incubation with fluorescein isothiocyanate-labeled goat anti-rabbit IgG (diluted 1:100) (MP Biomedicals, Irvine, CA) for 60
min. Immunofluorescence in the arteries was observed under a confocal laser-scanning microscope (CLSM 510; Carl Zeiss GmbH, Jena, Germany) in the Okayama University Medical School Central Research Laboratory. Control immunohistochemical staining for nNOS blocking peptide was done by preadsorbing the blocking peptide for primary antibody to nNOS (rat) (5 μg/ml; Cayman Chemical, Ann Arbor, MI) and exhausting the nNOS antibody with the relevant peptides.

**Statistics.** All values were expressed as mean ± S.E.M. Statistical analysis was evaluated using one-way analysis of variance followed by Tukey's test. A value of \( P < 0.05 \) was considered statistically significant.

**Drugs.** The following drugs were used: ACh chloride (Daichi Pharmaceutical Co., Tokyo, Japan), capsaicin (Sigma-Aldrich), guanethidine sulfate (Tokyo Kasei, Tokyo, Japan), human CGRP(8-37) (Peptide Institute, Osaka, Japan), l-arginine (Sigma-Aldrich), l-NAME (Sigma-Aldrich), l-NNA (Sigma-Aldrich), l-VNIO (Alexis Corporation, Läufelfingen, Switzerland), NE hydrochloride (Daichi-Sankyo, Tokyo, Japan), methoxamine hydrochloride (Nihon Shinyaku Co., Kyoto, Japan), papaverine hydrochloride (Sigma-Aldrich), rat CGRP (Peptide Institute), and SD (Ishizu Seiyaku, Tokyo, Japan). All drugs, except for capsaicin and SD, were dissolved in pure water and diluted with Krebs' solution. Capsaicin was dissolved in 50% ethanol and diluted with Krebs' solution (final alcohol concentration, 0.4 mg/ml). SD was dissolved in 0.9% saline. ACh and rat CGRP was diluted with Krebs' solution containing 2 μM methoxamine and 5 μM guanethidine when injected directly.

**Results**

**Effects of l-NAME, l-NNA, and l-VNIO on Vasoconstrictor Responses to PNS and NE Injection.** As shown in Fig. 1A, PNS (2, 4, and 8 Hz) of rat perfused mesenteric vascular beds without endothelium and with resting tone frequency-dependently increased the perfusion pressure due to vasoconstriction: 2 Hz, 6.7 ± 0.7 mm Hg (\( n = 5 \)); 4 Hz, 11.7 ± 1.0 mm Hg (\( n = 5 \)); and 8 Hz, 37.2 ± 4.6 mm Hg (\( n = 5 \)). Bolus injection of NE (0.5 or 1 nmol) into the perfusate also caused concentration-dependent vasoconstriction: 0.5 nmol, 14.1 ± 1.7 mm Hg (\( n = 5 \)); and 1 nmol, 25.4 ± 2.9 mm Hg (\( n = 5 \)) (Figs. 1A and 2A). Repeated PNS and NE injection caused reproducible vasoconstrictor responses. In the control response, the ratios of \( S_1 \) and \( S_2 \) at 2, 4, and 8 Hz and \( I_1 \) and \( I_2 \) at 0.5 and 1 nmol were 1.01 ± 0.09, 1.05 ± 0.05, 1.04 ± 0.03, and 1.19 ± 0.10, 1.20 ± 0.08, respectively (Fig. 2A). At the end of the experiment, the preparation was perfused with Krebs' solution containing methoxamine, a selective \( \alpha_1 \) adrenoceptor agonist, to increase vascular tone and guanethidine to block adrenergic neurotransmission. Chemical removal of the endothelium was confirmed by the lack of a relaxant effect after a bolus injection of 1 nmol ACh. At the end of each panel, the active tone of preparations was increased by methoxamine in the presence of guanethidine and chemical removal of the endothelium was demonstrated by verifying the lack of a relaxant effect after a bolus injection of 1 nmol ACh. Also, the CGRPergic nerve-mediated vasodilation was confirmed by applying 2 Hz PNS. Solid inverted triangles, PNS. Solid squares, bolus injections of NE. SD, perfusion of sodium deoxycholate.

**Fig. 1.** Typical records showing effects of NOS inhibitors on vasoconstrictor responses to PNS (2, 4, and 8 Hz) and bolus injections of NE (0.5 and 1.0 nmol) in rat perfused mesenteric vascular beds with resting tone and without endothelium. A, control responses in the absence of NOS inhibitor. B, responses in the presence of l-NAME. C, responses in the presence of l-VNIO. \( S_1 \) and \( S_2 \), responses to the first and second PNS. \( I_1 \) and \( I_2 \), responses to the first and second bolus injections of NE. Solid circle, bolus injection of ACh. At the end of each panel, the active tone of preparations was increased by methoxamine in the presence of guanethidine, and chemical removal of the endothelium was demonstrated by verifying the lack of a relaxant effect after a bolus injection of 1 nmol ACh. Also, the CGRPergic nerve-mediated vasodilation was confirmed by applying 2 Hz PNS. Solid inverted triangles, PNS. Solid squares, bolus injections of NE. SD, perfusion of sodium deoxycholate.

As shown in Fig. 1, B and C, in perfused mesenteric vascular beds without endothelium, perfusion of a competitive inhibitor of NO synthase, l-NAME (100 μM) or l-VNIO (100 μM), did not alter the resting tone. In the presence of l-NAME (1–100 μM) or L-VNIO (10–100 μM), vasoconstrictor responses to PNS at 2, 4, and 8 Hz were significantly augmented (Figs. 1B and 2A and Figs. 1C and 2C). l-NNA (1–100 μM) also caused concentration-dependent augmentation of the PNS-induced vasoconstriction (Fig. 2B). l-NAME, l-NNA, or l-VNIO did not affect the vasoconstrictor responses to exogenously applied NE (Figs. 1 and 2).

**Effect of l-Arginine on Vasoconstrictor Responses to PNS and NE Injection in the Presence of l-NAME.** As shown in Fig. 3A, the augmentation of PNS-induced vasoconstriction induced by l-NAME was reversed by additional perfusion with l-arginine (10 μM to 1 mM) (Fig. 3B). However, l-arginine did not affect vasoconstrictor responses to NE injection (Fig. 3C).

**Effect of Capsaicin Treatment on Vasoconstrictor Responses to PNS and NE Injection in the Presence of l-NAME.** In perfused mesenteric vascular beds, perfusion of 5 μM capsaicin did not alter the resting tone. As shown in Fig. 4, in preparations treated with capsaicin, l-NAME perfusion did not cause augmentation of vasoconstrictor responses to PNS or NE.

**Measurement of NE in the Perfusate.** As shown in Fig. 5A, in rat perfused mesenteric vascular beds without endo-
The basal release of NE was 7.13 ± 2.87 pg/ml (n = 8). The application of PNS at 8 Hz induced a significant increase in the amount of NE (30.68 ± 5.77 pg/ml, n = 5) in the perfusate. The perfusion of L-NAME (100 μM) significantly enhanced the PNS-evoked NE release (Fig. 5).

In preparations treated with capsaicin, the basal release of NE was 7.30 ± 3.64 pg/ml (n = 6). The first PNS after the treatment evoked greater NE release (49.48 ± 3.45 pg/ml) than that in control preparations without capsaicin treatment. There was a significant difference (P < 0.01) between the capsaicin-treated and control preparations in the net release of NE (pre-PNS minus post-PNS). As shown in Fig. 5, perfusion of L-NAME (100 μM) did not alter the PNS-evoked NE release in preparations treated with capsaicin.

**Effects of L-NAME, CGRP(8-37), or the Combination of L-NAME and CGRP(8-37) on Vasoconstrictor Responses to PNS and NE Injection.** Perfusion of CGRP(8-37) significantly augmented the vasoconstrictor response to PNS without affecting the NE-induced vasoconstriction. CGRP(8-37) perfusion in the presence of L-NAME caused further, additive augmentation of the PNS-induced vasoconstriction (Table 1). However, the combination of L-NAME and CGRP(8-37) did not affect the NE-induced vasoconstriction (Table 1).

**Effect of L-NAME on Vasodilation in Response to PNS and CGRP Injection.** In perfused mesenteric vascular beds without endothelium and with active tone, PNS (1 and 2 Hz) induced a frequency-dependent decrease in perfusion pressure due to vasodilation. A bolus injection of CGRP (10 pmol) into the perfusate also caused vasodilation. Repeated PNS and CGRP injections caused reproducible vasoconstrictor responses. In the control response, the ratios of S1 and S2 responses to the first and second PNS. I1 and I2 responses to the first and second bolus injections of NE. SD, perfusion of sodium deoxycholate. B and C, ordinates show the ratio of S1- and S2-induced vasoconstriction and the ratio of I1- and I2-induced vasoconstriction. Values represent the mean ± S.E.M. of five rats. *P < 0.05; **P < 0.01 compared with control.

**Immunohistochemical Study.** As shown in Fig. 7A, the mesenteric artery had dense innervation of nNOS-like
immunoreactivity (LI)-positive nerves. However, preadsorption with a blocking peptide for the primary antibody to nNOS (rat) resulted in the detection of little or no immunoreactivity (Fig. 7B). In the mesenteric artery treated with capsaicin, nNOS-LI-positive nerve fibers were not observed (Fig. 7C).

**Discussion**

**Augmentation of Adrenergic Nerve-Mediated Vasconstriction by NOS Inhibitors.** It has been reported that vasoconstriction in response to PNS of the mesenteric artery is abolished by tetrodotoxin (neurotoxin), guanethidine (adrenergic neuron blocker), prazosin (α₁-adrenoceptor antagonist), and 6-hydroxydopamine (adrenergic neuron destroyer) (Kawasaki and Takasaki, 1984; Kawasaki et al., 1987). Therefore, it is very likely that NE released from periarterial sympathetic adrenergic nerves mediates the PNS-induced vasoconstriction. This notion is confirmed by the present finding that PNS of the mesenteric artery increases the release of NE in the perfusate. The present study demonstrated that nonselective NOS inhibitors, L-NAME and L-NNA, augmented the vasoconstrictor response to PNS of rat mesenteric arteries without endothelium. Additionally, a selective nNOS-specific inhibitor, L-VNIO (Babu and Griffith, 1998), also augmented the vasoconstrictor response to PNS. However, these NOS inhibitors did not affect
the vasoconstriction in response to exogenously applied NE, suggesting that the augmentation of the PNS response induced by NOS inhibitors was not due to increased NE-induced vasoconstriction at postsynaptic sites. These results are consistent with the previous report that an NOS inhibitor (L-NNA) augmented the vasoconstrictor responses to sympathetic stimulation without endothelium (Rabelo et al., 2001). Therefore, it is very likely that NOS inhibitors enhance the release of the adrenergic neurotransmitter NE from perivascular adrenergic nerves. This notion is supported by the present finding that L-NAME significantly increased the release of NE evoked by PNS.

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PNS 4 Hz</th>
<th>PNS 8 Hz</th>
<th>NE 0.5 nmol</th>
<th>NE 1.0 nmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.12 ± 0.02</td>
<td>1.09 ± 0.02</td>
<td>1.08 ± 0.03</td>
<td>1.15 ± 0.02</td>
</tr>
<tr>
<td>CGRP(8-37)</td>
<td>1.70 ± 0.12*</td>
<td>1.74 ± 0.08**</td>
<td>1.11 ± 0.09</td>
<td>1.18 ± 0.05</td>
</tr>
<tr>
<td>L-NAME</td>
<td>1.38 ± 0.13*</td>
<td>1.54 ± 0.15*</td>
<td>1.15 ± 0.04</td>
<td>1.10 ± 0.01</td>
</tr>
<tr>
<td>CGRP(8-37) ± L-NAME</td>
<td>2.16 ± 0.26**</td>
<td>2.65 ± 0.23**</td>
<td>1.20 ± 0.05</td>
<td>1.26 ± 0.05</td>
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* P < 0.05 compared with control.
** P < 0.01 compared with control.
Involvement of Neuronal NO. L-Arginine completely reversed the enhancement by an NOS inhibitor (L-NAME) of the adrenergic nerve-mediated response, whereas L-arginine did not affect the vasoconstrictor response to exogenously applied NE. These findings strongly suggest that endogenous NO modulates the neurogenic release of NE from adrenergic nerve terminals. In the guinea pig pulmonary artery, NO has been shown not to affect the PNS-induced NE release (Brassai et al., 2002). In contrast, Barnes et al. (2001) reported that NO, which is released from neuronal and endothelial sources, modulates the evoked catecholamine release from the canine adrenal medulla. In the present study, an NOS inhibitor, L-NAME, facilitated PNS-induced NE release, suggesting that endogenous NO presynaptically inhibits the neurogenic release of NE in rat mesenteric arteries.

Involvement of Capsaicin-Sensitive Nerves. In the present study, L-NAME did not augment the pressor response to PNS when the rat mesenteric artery was treated with capsaicin, a depletor of primary sensory nerves. Additionally, capsaicin treatment inhibited the L-NAME-induced facilitation of NE release evoked by PNS. Primary sensory nerve fibers containing NOS are distributed in the inferior mesenteric ganglion in the guinea pig (Zheng et al., 1999). Furthermore, the present immunohistochemical study showed the presence of capsaicin-sensitive nNOS-LI-positive nerves in the mesenteric artery. This finding suggests that NOS is present in perivascular sensory nerves. The present study showed that pretreatment with capsaicin abolished the L-NAME-induced augmentation of adrenergic vasoconstriction in response to PNS. Since the endothelium of the mesenteric artery had been removed, it is possible that the source of NO was capsaicin-sensitive nerves. We previously reported that PNS of the rat mesenteric artery induces neurogenic vasodilation, which is abolished by pretreatment with capsaicin or CGRP(8-37), a C-terminal fragment of CGRP that is a CGRP receptor antagonist, suggesting that endogenous CGRP is released from CGRPergic nerves and induces vasodilation of the rat mesenteric artery (Kawasaki et al., 1991). Therefore, it seems likely that the NO involved in the presynaptic inhibition of adrenergic nerves might be released from capsaicin-sensitive CGRPergic nerves. This may explain the present finding that capsaicin treatment facilitated the PNS-induced NE release compared with that in nontreated preparations.

Interaction with CGRPergic Nerves. Oroszi et al. (1999) reported interplay between NO and CGRP in the isolated guinea pig heart. Furthermore, Gumusel et al. (2001) reported that NO mediates NANC relaxation in the rat pulmonary artery and inhibits the release of NANC neurotransmitter(s), which mediate the residual relaxation. In the present study, L-NAME in the preconstricted mesenteric artery without endothelium did not alter the PNS-induced vasodilation, which is mediated by CGRPergic nerves. In contrast, Ralevic (2002) reported that L-NAME has a postjunctional effect of inhibiting sensory neurogenic vasorelaxation in the absence of endothelium. However, the present findings showed that L-NAME did not attenuate the vasodilator response to exogenous CGRP, suggesting that L-NAME has no effect on CGRPergic vasodilation. Furthermore, the present results demonstrated that the presence of L-NAME together with CGRP(8-37) caused additive augmentation of the PNS-induced vasoconstrictor responses that were obtained with L-NAME or CGRP(8-37) alone. These results suggest that NO, which is released from perivascular capsaicin-sensitive nerves (probably CGRPergic nerves) is responsible for the presynaptic inhibition of adrenergic nerve neurotransmission but is not involved in the vasodilator response to PNS.

Conclusions
In conclusion, the present results suggest that NO, which is released from capsaicin-sensitive perivascular nerves, presynaptically inhibits neurogenic NE release to modulate...
adrenergic neurotransmission in rat mesenteric arteries (Fig. 8). Our findings also suggest that neurogenic NO in the mesenteric artery is involved in the presynaptic modulation of adrenergic neurotransmission. However, as illustrated in Fig. 8, the present findings do not distinguish between whether NO is released from NO-containing nerves or CGRPergic nerves that are colocalized with NO.

**References**


**Address correspondence to:** Dr. Hiromu Kawasaki, Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan. E-mail: kawasaki@pheasant.pharm.okayama-u.ac.jp