

Role of Endothelium-derived Hyperpolarizing Factor in Phenylephrine-induced Oscillatory Vasomotion in Rat Small Mesenteric Artery

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Background: In small mesenteric arteries, endothelium-derived hyperpolarizing factor (EDHF) in addition to endothelium-derived relaxing factors (EDRFs) including NO plays an important role in acetylcholine-induced vasodilation. It has been reported that EDRFs play an important role in α_1 -adrenoceptor agonist-induced oscillatory vasomotion and in limiting vasoconstrictor response to the agonists; however, contribution of EDHF to the α_1 -agonist-induced oscillation is unknown.

Methods: Rat small mesenteric arteries were isolated and cannulated at each end with a glass micropipette. The vessels were immersed in a bath (37°C) containing physiologic saline solution. Changes in vessel diameter were measured using an optical density video detection system.

Results: Denudation of the endothelium and inhibition of NO synthesis caused a leftward shift in the concentration-response relation for phenylephrine in the mesenteric arteries, whereas inhibition of cyclooxygenase by indomethacin had no effect. Blockade of Ca^{2+} -activated K^+ (K_{Ca}) channels by charybdotoxin and apamin caused a further leftward shift in the concentration-response relation in the vessels pretreated with N^{ω} -nitro-L-arginine methylester and indomethacin. Phenylephrine at concentrations higher than 10^{-6} M caused endothelium-dependent oscillatory vasomotion, which was reduced but not abolished after combined inhibition of the cyclooxygenase and NO synthase pathways. However, the K_{Ca} channel blockers completely abolished the remaining component of oscillation.

Conclusions: Endothelially-derived NO is an important modulator of sustained agonist-induced vasoconstriction. NO, as well as endothelially-derived cyclooxygenase products and EDHF, also contribute significantly to phenylephrine-induced oscillatory vasomotion.

VASOCONSTRICTOR response of small mesenteric arteries to α_1 -adrenoceptor stimulation results in reduction in intestinal microcirculation.¹ However, endothelium-derived relaxing factors (EDRFs) such as NO have been shown to counterbalance or attenuate the α_1 -adrenoceptor-mediated constriction.¹⁻⁴ In rat small mesenteric arteries, the contractile responses to sympathetic nerve stimulation^{1,2} and α_1 -adrenoceptor agonists^{3,4} are augmented by denudation of the endothelium or inhibition of NO synthase. The role of NO as an important modulator of α_1 -adrenergic constriction has also been demon-

strated in the rat muscle^{5,6} and canine pulmonary⁷ arteries. Therefore, NO may contribute to maintenance of an adequate blood supply to organs during stimulation of the sympathetic nervous system. The α_1 -adrenoceptor agonists induce rhythmic oscillatory vasomotion in small mesenteric arteries.^{4,8,9} This oscillation may also provide advantages in the local flexible control of tissue perfusion during sympathetic activation.⁸ Gustafsson *et al.*⁹ reported that α_1 -agonist-induced oscillation was mediated by endothelium-derived NO, since norepinephrine-induced oscillatory vasomotion was abolished by inhibition of the endothelium-dependent relaxation system.

Endothelium-derived hyperpolarizing factor (EDHF) is known as another mediator of endothelium-dependent vasodilation. In acetylcholine-induced vasodilation, EDHF in addition to NO plays an important role in small mesenteric arteries.¹⁰ Recent study illustrates that phenylephrine, a selective α_1 -adrenoceptor agonist, induced oscillatory vasomotion, which remained after inhibition of NO synthase.⁴ This finding suggests that EDHF plays a role in the α_1 -agonist-induced oscillation. However, little is known about the attenuating effect of EDHF on the vasoconstrictor response to α_1 -adrenoceptor agonists.

The purpose of this study was to compare the influence of endothelially-released NO, cyclooxygenase products and EDHF on modulating phenylephrine-induced vasoconstriction and oscillatory vasomotion in *in vitro* perfused mesenteric arteries.

Materials and Methods

Vessel Preparation

With approval from the Sapporo Medical University Animal Care and Use Committee, male Sprague-Dawley rats (6-9 weeks old) were anesthetized with ether and exsanguinated by hemorrhage. A section of the mesentery 5-10 cm distal to the pylorus was rapidly removed and placed in oxygenated cold physiologic saline solution (PSS). Segments of third or fourth branches of mesenteric arteries (~200 μm maximum diameter) were carefully isolated and removed the connective tissues.

Diameter Measurement Experiment

Using an optical density video detection, we measured changes in internal diameters of vessels with controlled intraluminal pressure. This method may be more suitable than other *in vitro* methods for investigation of physiologic vasomotion.¹¹

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Received from the Department of Anesthesiology, Sapporo Medical University School of Medicine, Sapporo, Japan. Submitted for publication September 20, 2002. Accepted for publication January 2, 2003. Support was provided solely from departmental sources.

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After each dissected vessel (~5 mm in length) was placed in a microvessel chamber filled with PSS, both the proximal and distal ends were cannulated with a glass micropipette and secured with 10-0 nylon sutures. After securing the proximal end, the lumen was gently perfused to flush and clear the vessel of clotted blood before securing the distal end. Then the PSS in the chamber was continually circulated from a reservoir in which the solution was aerated with a 95% O₂– 5% CO₂ mixture. The volume of the chamber and reservoir was 100 ml, and the rate of flow of the suffusing solution was 15 ml/min. The PSS was heated before passing into the chamber in order to maintain the bath temperature at 37°C using an automatic temperature controller (TC-324B, Warner Instrument, Hamden, CT). The chamber was placed on the stage of an inverted microscope (IX70, Olympus, Tokyo, Japan) connected to a video camera (WAT-308A, WATEC, Yamagata, Japan), and the vessel image was projected onto a television screen (Sony, Tokyo, Japan). The changes in vessel internal diameters were measured using a video dimension analyzer system (Living Systems Instrumentation, Burlington, VT). Measurements of diameters were recorded using a personal computer connected to the analyzer.

Experimental Protocols

Intraluminal pressure was kept constant at 50 mmHg by a pressure servo-control pump in a no-flow state to exclude the influence of shear stress. After a 30-min equilibration period, each vessel was initially constricted with 60 mM potassium solution (equimolar substitution of KCl by NaCl in PSS). After removal of the high-potassium solution and return of the diameters to prestimulation values, phenylephrine concentration-response relationships under various conditions were obtained for each vessel by increasing the concentration of phenylephrine in half-log increments (10^{-8} to 3×10^{-5} M) after the response to each preceding concentration had reached a steady state.

Protocol 1: Effects of Endothelium Denudation on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion. First, we compared the responses to phenylephrine in vessels with and without endothelium to assess the roles of endothelium function in the modulation of phenylephrine-induced vasoconstriction and oscillatory vasomotion. The endothelium was removed by inserting a hair into the lumen before the cannulation with micropipettes. Removal of the endothelium was verified by loss of vasodilator response to 10^{-6} M of acetylcholine after administration of the highest concentration of phenylephrine.

Protocol 2: Effects of α_1 -Adrenoceptor Antagonist on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion. To confirm that the phenylephrine-induced constriction is mediated by α_1 -adreno-

ceptor activation, endothelium-intact vessels were incubated with an α_1 -adrenoceptor antagonist, prazosin (10^{-9} M).

Protocol 3: Effects of Inhibitors of Endothelium-derived Mediators on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion. A third series of experiments was performed to identify the specific endothelium-derived mediators involved in the modulation of vasoconstrictor response and the oscillatory vasomotion. For 30 min before the administration of phenylephrine, endothelium-intact vessels were incubated with one or both of the following pharmacologic inhibitors: *N*^ω-nitro-L-arginine methylester (L-NAME; 10^{-4} M), an inhibitor of NO synthase, and indomethacin (3×10^{-5} M), an inhibitor of cyclooxygenase.

Protocol 4: Effects of EDHF Inhibition on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion. To assess the role of EDHF in phenylephrine-induced oscillatory vasomotion, a fourth series of experiments was performed using endothelium-intact vessels in the presence of indomethacin (3×10^{-5} M) and L-NAME (10^{-4} M).

Although the nature of EDHF is still unresolved, recent evidences indicate that activation of the small and intermediate conductance Ca²⁺-activated K⁺ (K_{Ca}) channels on endothelial cells is one of the proposed mechanisms regulating EDHF release.^{4,12-17} Therefore, the intralumens of endothelium-intact vessels were incubated with a combination of apamin (5×10^{-7} M, an inhibitor of small conductance K_{Ca} channels) and charybdotoxin (10^{-7} M, an inhibitor of intermediate conductance K_{Ca} channels) for 30 min before the administration of phenylephrine. In this experiment, apamin and charybdotoxin were perfused intraluminally to apply to the endothelium, and intraluminal pressure was maintained at 45 mmHg by hydrostatic pressure.

Solutions and Drugs

The PSS comprised the following: NaCl, 119 mM; KCl, 4.7 mM; NaHCO₃, 25 mM; CaCl₂, 2.5 mM; KH₂PO₄, 1.18 mM; MgSO₄, 1.17 mM; glucose, 5.5 mM; EDTA, 0.026 mM. The following drugs and chemicals were used: phenylephrine, acetylcholine chloride, indomethacin, L-NAME, prazosin, apamin and charybdotoxin (Sigma Chemical, St. Louis, MO). All drug concentrations are expressed as final molar concentrations in the vessel chamber. Indomethacin was dissolved in ethanol (100%) and then diluted in distilled water to obtain the desired concentrations (final vessel chamber ethanol concentration was 0.075%). All other drugs were dissolved in distilled water.

Data Analysis

Values are expressed as the mean \pm SD. Responses to phenylephrine are expressed as a percentage of the

vasoconstrictor response to 60 mM potassium solution. When endothelium-intact vessels exhibited oscillatory vasomotion, both maximum and minimum values were plotted. Amplitude of the phenylephrine-induced oscillatory vasomotion was calculated by subtracting the minimum value from the maximum value. The effects of denudation and the inhibitors on the concentration-response curves to phenylephrine were assessed by calculating the concentration of phenylephrine causing 50% of the maximal response (ED_{50}). This value was interpolated from the linear portion of the concentration-response curve by regression analysis and is presented as $\log ED_{50}$. Statistical analyses of the data were performed using the two-tailed Student *t* test for unpaired comparison. When more than two mean values were compared, analysis of variance and the Tukey-Kramer test were used. Values were considered to be significant when *P* was less than 0.05.

Results

Effects of Endothelium Denudation on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion

The mean baseline diameter of endothelium-intact vessels was not significantly different from the mean baseline diameter of endothelium-denuded vessels. Phenylephrine caused vasoconstriction in a concentration-dependent manner in both endothelium-intact and endothelium-denuded vessels (figs. 1 and 2). However, denudation of the endothelium significantly ($P < 0.05$) decreased the ED_{50} for phenylephrine ($\log ED_{50} = -6.46 \pm 0.03$, $n = 6$) compared to that in endothelium-intact vessels ($\log ED_{50} = -6.07 \pm 0.03$, $n = 6$), indicating that the endothelium functioned as a modulator of phenylephrine-induced vasoconstriction. As shown in figure 1A, endothelium-intact vessels exhibited oscillatory vasoconstriction at concentrations of phenylephrine higher than 10^{-6} M. In contrast, endothelium-denuded vessels showed a steady response to phenylephrine, without any rhythmic activity (fig. 1B). Figure 2B illustrates the amplitude of the oscillation at 10^{-5} M phenylephrine. The endothelium denudation completely abolished the phenylephrine-induced oscillation. The peak of oscillatory vasomotion at 10^{-5} M phenylephrine in endothelium-intact vessels ($105 \pm 8\%$) was equal to tonic vasoconstriction in endothelium-denuded vessels ($105 \pm 3\%$). Therefore, it is suggested that this oscillatory vasomotion is due to endothelium-dependent vasodilator mechanisms.

Effects of α_1 -Adrenoceptor Antagonist on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion

Baseline diameters were not significantly different before and after incubation of the vessels with prazosin, an

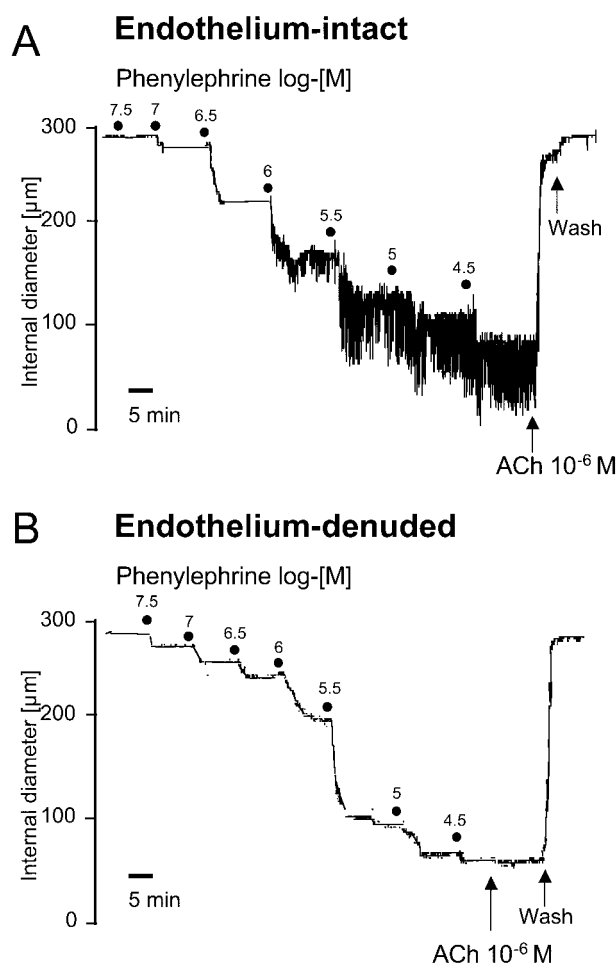


Fig. 1. Typical examples of changes in internal diameters of an endothelium-intact vessel (A) and an endothelium-denuded vessel (B). Note that the endothelium-intact vessel exhibited oscillatory vasomotion at concentrations of phenylephrine higher than 10^{-6} M, whereas the endothelium-denuded vessel showed only tonic constriction. Successful endothelium denudation was confirmed by a reduced dilatory response to 10^{-6} M acetylcholine (ACh).

α_1 -adrenoceptor antagonist. Prazosin caused a parallel rightward shift in the phenylephrine concentration-response curve (fig. 3A) and significantly ($P < 0.05$) increased the ED_{50} ($\log ED_{50} = -5.82 \pm 0.16$, $n = 5$) compared to the control curve and ED_{50} ($\log ED_{50} = -6.12 \pm 0.03$, $n = 5$). This finding indicates that the vasoconstrictor response to phenylephrine is selectively mediated by α_1 -adrenoceptor activation. As shown in figure 3B, prazosin also shifted the concentration of phenylephrine at which the oscillatory vasomotion was initiated (10^{-6} M in control vessels and 3×10^{-6} M in prazosin-incubated vessels). At concentrations of phenylephrine higher than 3×10^{-6} M, there were no significant differences between the amplitudes of oscillation in control and prazosin-treated vessels (fig. 3B). Therefore, it is suggested that the phenylephrine-induced oscillation is mediated by α_1 -adrenergic receptors.

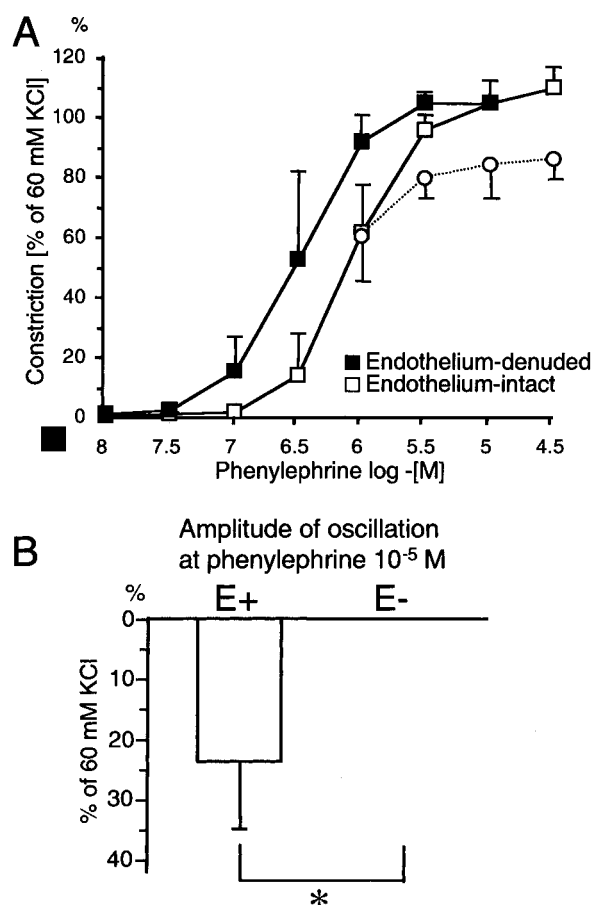


Fig. 2. Effect of endothelium denudation on vasoconstrictor response to phenylephrine. (A) Vasoconstrictions are expressed as percentages of 60 mM KCl constriction and are presented as the mean \pm SD. When the vessels exhibited the oscillation, both maximum (squares and solid line) and minimum (circles and dotted line) values were plotted. Endothelium denudation caused leftward shift in the phenylephrine concentration-effect curve and abolished the oscillation ($n = 6$). (B) Effects of endothelium denudation on the amplitude of 10^{-5} M phenylephrine-induced oscillation are summarized ($n = 6$). *Significantly different from control ($P < 0.05$).

Effects of NO Synthase Inhibition and Cyclooxygenase Inhibition on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion

The effects of indomethacin and L-NAME, alone and in combination, on phenylephrine-induced vasoconstriction are summarized in figure 4A, B, and C. Baseline diameters were not significantly different before and after incubation of the vessels with these inhibitors. Indomethacin alone did not alter phenylephrine-induced vasoconstriction (fig. 4A). In contrast, in the vessels incubated with L-NAME alone, a greater constriction was observed for the same dose of phenylephrine (fig. 4B), and the ED_{50} was significantly ($P < 0.05$) decreased (log $ED_{50} = -6.31 \pm 0.09$, $n = 5$) compared with the control value (log $ED_{50} = -6.11 \pm 0.08$, $n = 5$). As shown in figure 4C, combined inhibition with L-NAME and indomethacin had no additional effect on the ED_{50} for phenylephrine (log $ED_{50} = -6.35 \pm 0.20$, $n = 5$).

The effects of the endothelial inhibitors on phenylephrine-induced oscillatory vasomotion are summarized in figure 5. Either indomethacin alone or L-NAME alone significantly decreased the amplitude of the oscillatory vasomotion at 10^{-5} M phenylephrine (fig. 5). A combination of both inhibitors also decreased, but did not abolish the oscillatory vasomotion (fig. 5).

These results suggest that endothelial modulation of phenylephrine-induced vasoconstriction is mediated by NO but not by cyclooxygenase products. On the other hand, the phenylephrine-induced oscillatory vasomotion may involve a cyclooxygenase-dependent, NO-dependent, and other mechanisms.

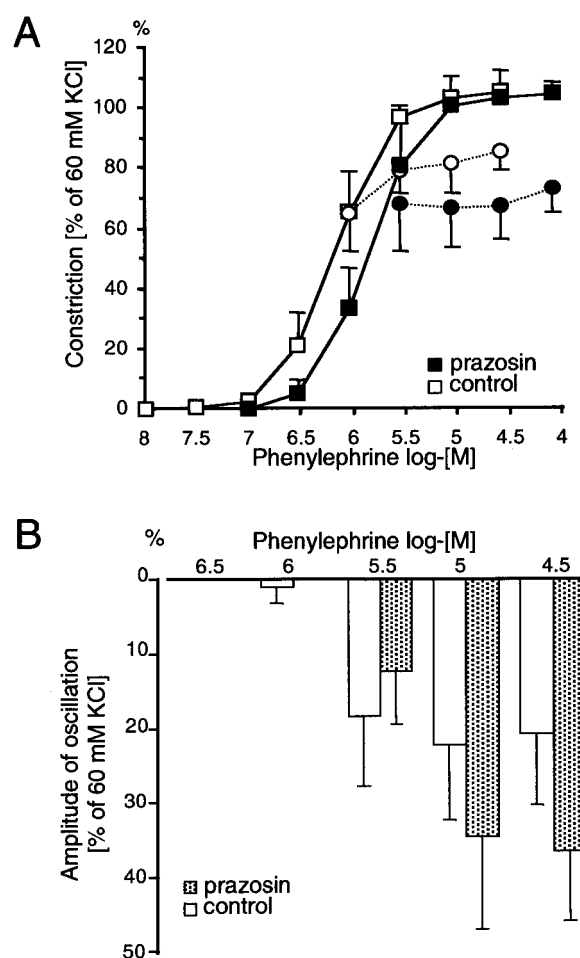


Fig. 3. Effect of the α_1 -adrenoceptor antagonist prazosin (10^{-9} M) on vasoconstrictor response to phenylephrine. (A) Vasoconstrictions are expressed as percentages of 60 mM KCl constriction and are presented as the mean \pm SD. When the vessels exhibit the oscillation, both maximum (squares and solid line) and minimum (circles and dotted line) values were plotted. Prazosin caused a rightward shift in the phenylephrine concentration-effect curve ($n = 5$). (B) Amplitudes of phenylephrine-induced oscillation are summarized as a function of concentrations of phenylephrine ($n = 5$).

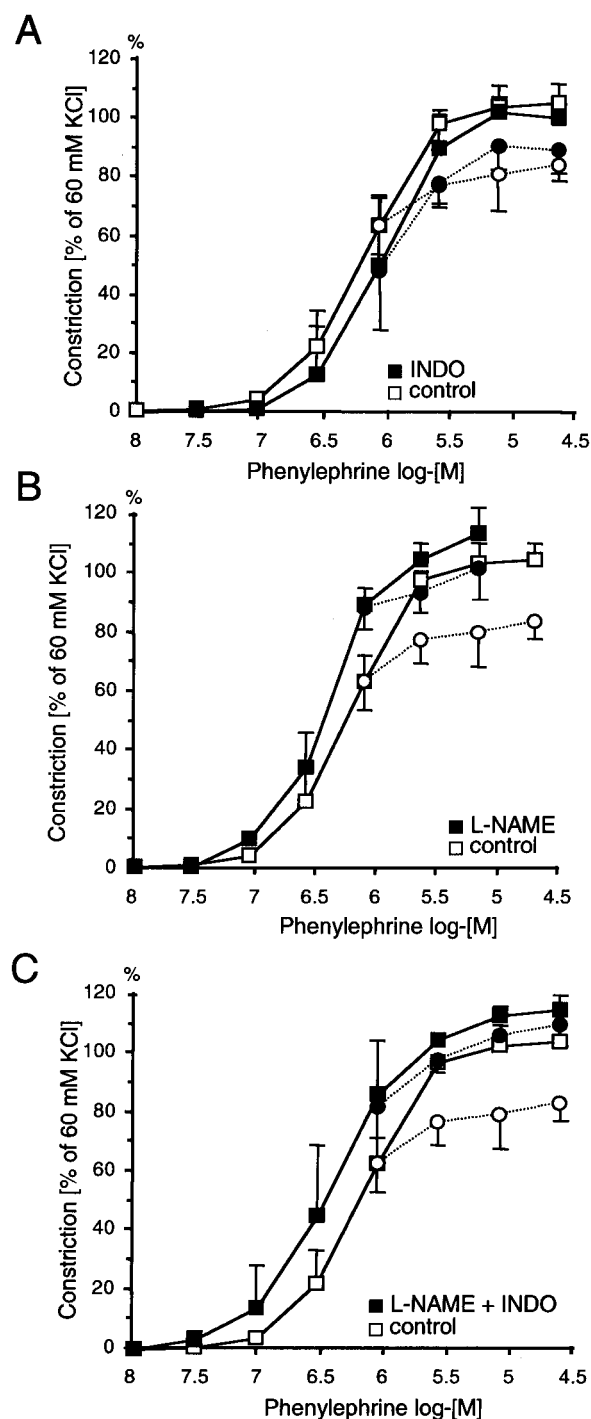


Fig. 4. Effects of (A) cyclooxygenase inhibition by indomethacin (INDO, 3×10^{-5} M), (B) NO synthase inhibition by L-NAME (10^{-4} M) and (C) combined inhibition on vasoconstrictor response to phenylephrine ($n = 5$). Vasoconstrictions are expressed as percentages of 60 mM KCl constriction and are presented as the mean \pm SD. When the vessels exhibited oscillation, both maximum (squares and solid line) and minimum (circles and dotted line) values were plotted.

Effects of EDHF Inhibition on Phenylephrine-induced Vasoconstriction and Oscillatory Vasomotion

In the vessels incubated with a combination of indomethacin and L-NAME, it is expected that EDHF is re-

sponsible for the remaining component of phenylephrine-induced oscillatory vasomotion. Since recent studies indicate that EDHF-mediated vasodilation depends on activation of K_{Ca} channels having small and intermediate conductance on endothelial cells,^{4,12-17} a combination of apamin and charybdotoxin was applied into the lumens of endothelium-intact vessels. Consistent with previous reports,^{14,18} this treatment did not alter the base-

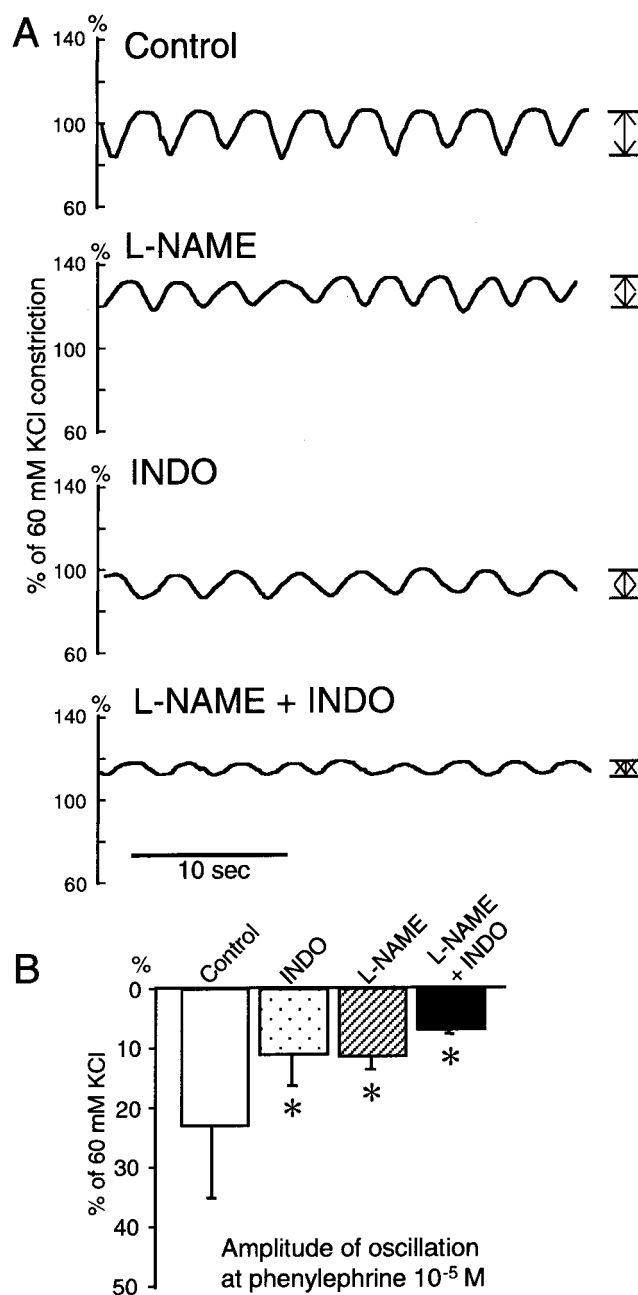
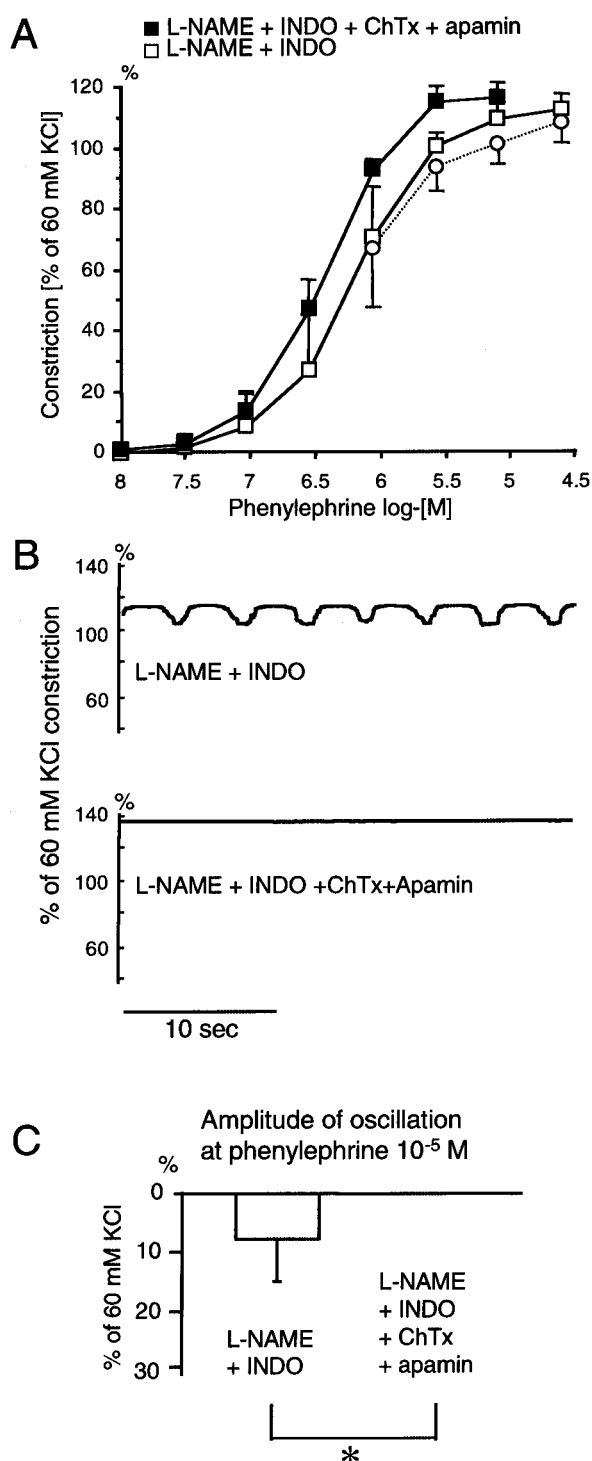


Fig. 5. (A) Typical records of oscillatory vasomotion at 10^{-5} M phenylephrine in vessels pretreated with no inhibitor (control), L-NAME (10^{-4} M) alone, indomethacin (INDO, 3×10^{-5} M) alone, and a combination of L-NAME and indomethacin. Arrows indicate amplitudes of oscillatory vasomotion. (B) Amplitude of 10^{-5} M phenylephrine-induced oscillation are summarized ($n = 5$). *Significantly different from control ($P < 0.05$).



line diameter. In these conditions, a greater constriction was observed for the same dose of phenylephrine (fig. 6A) and the ED_{50} was significantly ($P < 0.05$) decreased ($\log ED_{50} = -6.46 \pm 0.10$, $n = 5$) compared to the control value ($\log ED_{50} = -6.19 \pm 0.10$, $n = 5$). The phenylephrine-induced oscillation was abolished completely by the K_{Ca} blockers (figs. 6B and 6C), indicating that the remaining component of oscillation in the pres-

Fig. 6. (A) Effect of blockade of Ca^{2+} -activated K^{+} channels by a combination of charybdotoxin (ChTx, 10^{-7} M) and apamin (5×10^{-7} M) on vasoconstrictor response to phenylephrine in vessels pretreated with a combination of indomethacin (INDO, 3×10^{-5} M) and L-NAME (10^{-4} M) ($n = 5$). Vasoconstrictions are expressed as percentages of 60 mM KCl constriction and are presented as the mean \pm SD. When the vessels exhibited oscillation, both maximum (squares and solid line) and minimum (circles and dotted line) values were plotted. (B) Typical traces of oscillatory vasomotion at 10^{-5} M phenylephrine in a vessel pretreated with a combination of indomethacin (INDO, 3×10^{-5} M) and L-NAME (10^{-4} M) and in a vessel pretreated with a combination of indomethacin (INDO, 3×10^{-5} M), L-NAME (10^{-4} M), charybdotoxin (ChTx, 10^{-7} M) and apamin (5×10^{-7} M). (C) Amplitude of 10^{-5} M phenylephrine-induced oscillation are summarized ($n = 5$). *Significantly different from control ($P < 0.05$).

ence of indomethacin and L-NAME was mediated by EDHF.

Discussion

Vascular endothelium has been implicated as important modulator of α -adrenergic constriction. The specific mechanisms through which the endothelium exerts this modulation may involve the release of NO,^{2-6,19,20} cyclooxygenase products,^{21,22} and EDHF.⁴ In the present study, NO synthase inhibition, but not cyclooxygenase inhibition, caused a leftward shift in the phenylephrine concentration-response curve. This indicates that NO is an important modulator of phenylephrine-induced constriction in the rat mesenteric artery. In contrast to the abundance of information on NO and cyclooxygenase products, little is known about the role of EDHF during α_1 -adrenoceptor-mediated vasoconstriction. Tuttle *et al.*⁶ reported that the presence of tetrabutylammonium, which has been thought to block K_{Ca} channels, did not alter the α -adrenergic vasoconstriction in rat skeletal muscle arteries. In the rat small mesenteric artery, Dora *et al.*⁴ reported that blockade of K_{Ca} channels by charybdotoxin and apamin increased the constrictor response to phenylephrine. Consistent with the latter report, we observed that a combination of charybdotoxin and apamin caused a leftward shift in the phenylephrine concentration-response curve. The present results suggest that not only NO but also EDHF is involved in the reduction of α_1 -adrenergic-stimulated tone in the rat small mesenteric artery.

Agonist-induced oscillatory vasomotion in small arteries is observed in most tissues and species and can be produced *via* both endothelium-independent and -dependent mechanisms.⁸ We demonstrated that phenylephrine at concentrations higher than 10^{-6} M induced spontaneous oscillatory vasomotion in an endothelium-dependent manner. The peak constriction during oscillation in the endothelium-intact vessels was equal to tonic constriction in the endothelium-denuded vessels,

suggesting that the oscillation is due to rhythmic vasodilatation mediated by an endothelium-dependent mechanism. Gustafsson *et al.*⁹ reported that norepinephrine-induced tension oscillation was abolished by NO synthesis inhibition in the rat small mesenteric artery, indicating that the oscillation is mediated mainly by release of NO from the endothelium. In the present study, however, we found that not only NO synthesis inhibition but also cyclooxygenase inhibition attenuated the oscillation. Furthermore, the oscillation still remained even after the combined inhibition of the NO synthase and cyclooxygenase pathways. The oscillation that remained was abolished by additional blockade of K_{Ca} channels by charybdotoxin and apamin. Therefore, besides the role of NO, these results indicate important roles of cyclooxygenase products and EDHF in the endothelium-dependent oscillation. Consistent with our findings, Dora *et al.*⁴ observed that the phenylephrine-induced oscillation that remained after NO synthase inhibition was abolished by K_{Ca} channel blockers in the rat small mesenteric artery, although they did not describe about that in detail. Furthermore, in the rabbit ear artery, phenylephrine-induced oscillation was inhibited by charybdotoxin but not by NO synthesis inhibition.²³ These studies support our speculation that EDHF is involved in the mechanisms of phenylephrine-induced oscillatory vasomotion.

The mechanisms of the release of endothelial vasodilators that contribute to phenylephrine-induced oscillation are still unknown. Since the release of endothelium-derived factors depends on the increase in endothelial $[Ca^{2+}]_i$, it is postulated that phenylephrine-induced elevation of endothelial $[Ca^{2+}]_i$ and consequent release of endothelial vasodilators may contribute to the oscillation. In fact, it has been reported that α_1 -adrenergic agonists, including norepinephrine⁶ and phenylephrine,^{3,18} increase endothelial $[Ca^{2+}]_i$ in isolated vessels. Although there is no evidence of the oscillatory release of endothelium-derived factors, tension oscillation accompanied by endothelial $[Ca^{2+}]_i$ oscillation was observed in the rat tail artery.²⁴ This may support the concept that a rise in endothelial $[Ca^{2+}]_i$ is key event for phenylephrine-induced oscillation. However, phenylephrine failed to directly increase $[Ca^{2+}]_i$ in endothelial cells freshly isolated from small mesenteric arteries.⁴ Therefore, it is suggested that the increase in $[Ca^{2+}]_i$ is due to indirect action of phenylephrine on endothelial cells. Recently, it has been suggested that a rise in vascular smooth muscle $[Ca^{2+}]_i$ stimulated by phenylephrine may diffuse to underlying endothelial cells through myoendothelial gap junctions.^{3,18} Tuttle *et al.*⁶ demonstrated that the vasoconstrictor prostaglandin $F_{2\alpha}$ did not increase endothelial $[Ca^{2+}]_i$ in rat muscle arteries. In this study, norepinephrine produced a large transient peak in vascular smooth muscle $[Ca^{2+}]_i$, whereas the vasoconstrictor prostaglandin $F_{2\alpha}$ produced a rise in vascular

smooth muscle $[Ca^{2+}]_i$ without a peak. In our preliminary experiments, prostaglandin $F_{2\alpha}$ induced a tonic vasoconstriction with only a small oscillation in rat small mesenteric arteries. Therefore, it is predicted that a large transient increase in vascular smooth muscle $[Ca^{2+}]_i$ will be followed by an elevation of endothelial $[Ca^{2+}]_i$ and consequent release of vasodilators, which may contribute to the oscillatory vasomotion. Many previous studies have indicated that α_2 - and β -adrenoceptors on endothelial cells may play a physiologic role in the regulation of vasomotor tone.²⁵ However, activation of these endothelial adrenoceptors by phenylephrine may not contribute to the oscillation mainly, because phenylephrine does not directly increase endothelial $[Ca^{2+}]_i$ in endothelial cells freshly isolated from small mesenteric arteries.⁴

The precise nature of EDHF is still a matter of great debate and may involve more than one factor. Cytochrome P450 metabolites (EETs) have been considered as potential candidates for EDHF.^{26,27} However, cytochrome P450 inhibition by SKF525A did not alter the amplitude of phenylephrine-induced oscillation in our preliminary experiments. Recently, many studies^{12,13,16,17,28} have suggested that a rise in endothelial $[Ca^{2+}]_i$ elicit opening of endothelial K_{Ca} channels and that the consequent hyperpolarization is conducted to smooth muscle *via* myoendothelial gap junctions. We speculate therefore that the EDHF-mediated component of phenylephrine-induced oscillation is secondary to increase in vascular smooth muscle $[Ca^{2+}]_i$. The Ca^{2+} diffusion from smooth muscle through myoendothelial gap junctions may elicit opening of endothelial K_{Ca} channels, and the consequent hyperpolarization conducted to smooth muscle *via* myoendothelial gap junctions may contribute to the EDHF-mediated component of oscillation.

To the extent that our findings in isolated small mesenteric arteries may apply in the intact intestinal circulation, the oscillatory vasomotion may provide advantages in the regional control of intestinal perfusion^{8,29} in patients with circulatory shock treated with a high dose of α_1 -adrenergic agonists. In addition, our finding of the EDHF-mediated oscillation may provide a suitable model for studying the effects of anesthetics on physiologic function of EDHF. In many previous studies, acetylcholine has been used to stimulate EDHF release; however, it is not a physiologic mediator of release of EDHF.

In conclusion, the endothelium plays an important role in modulation of phenylephrine-induced vasoconstriction in the rat mesenteric artery. Phenylephrine at concentrations higher than 10^{-6} M produces endothelium-dependent oscillatory vasomotion, which is partly mediated by EDHF.

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