The Role of Endothelium in the Phenylephrine-Induced Oscillatory Responses of Rabbit Mesenteric Arteries

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ABSTRACT—Phenylephrine-induced oscillatory contractions in rabbit mesenteric arteries were investigated in vitro. Adrenergic, cholinergic, or histamine antagonists as well as cyclooxygenase and lipoxygenase inhibitors had no effect on this phenylephrine-induced oscillation. The removal of extracellular calcium ions or treatment with a calcium antagonist reduced the amplitude and frequency of the oscillation. Removal of the endothelium or treatment with inhibitors of the synthesis or the target enzyme of endothelium-derived relaxing factor (EDRF) also reduced the amplitude and frequency of the oscillation. In a perfusion bioassay, the perfusate from an endothelium-intact arterial segment induced oscillation of an endothelium-denuded arterial ring recipient. These results suggest that phenylephrine-induced oscillation is mediated by an endothelium-derived factor such as EDRF and depends on the influx of extracellular calcium ions.

Keywords: Phenylephrine-induced oscillation, Endothelium, Mesenteric artery (rabbit), EDRF, Extracellular calcium ion

Agonist-induced or spontaneous rhythmic oscillations have been observed in various isolated vessels such as hog carotid artery (1), human coronary artery (2–4), rat mesenteric artery (5), rat aorta (6), dog paw subcutaneous artery (7), dog renal vein (8), cat submucosal arteriole (9), and hypertensive rat femoral artery (10). In some studies, the oscillations were considered to be myogenic in origin.

Since endothelial cells may release both relaxing factors (11) and contracting factors (12), the contraction or relaxation of vascular smooth muscle induced by some agents has been found to depend on or be modified by these endothelium-derived factors (13, 14).

There is conflicting evidence regarding the contribution of endothelium to the oscillatory responses. The oscillatory responses induced by phenylephrine or prostaglandin F2α in hamster aortas have been shown to depend on the presence of endothelial cells (15, 16). In contrast, it has been reported that the oscillations induced by norepinephrine in tail arteries of spontaneously hypertensive rats are not affected by removal of the endothelium (17).

Although noradrenaline has been shown to evoke oscillatory responses in rabbit mesenteric arteries (18–22), the exact mechanism involved, including the contribution of the endothelium to this oscillation, remains unclear. The present study was performed to elucidate the mechanism of phenylephrine-induced oscillation in rabbit mesenteric arteries using organ bath studies and perfusion bioassay. It was found that the oscillation induced by stimulation with this α1-agonist depended on the presence of the endothelium and was mediated by endothelium-derived factor.

MATERIALS AND METHODS

Organ bath studies
Male albino rabbits (3.1–3.4 kg) were anesthetized with sodium pentobarbital (20 mg/kg, i.v.) and exsanguinated. The mesenteric artery was immediately removed from each rabbit, cleaned of adherent connective tissue, and cut into approximately 2.5-mm wide rings. In some experiments, the endothelium was removed by rubbing the intimal surface of the artery with a roughened needle. Each arterial ring was mounted on a specially designed holder and incubated in a 5-ml organ bath containing oxygenated (95% O2, 5% CO2) Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl,
2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 10 mM glucose) and maintained at 37°C. The arterial rings were equilibrated for more than 1 hr at a resting tension of 0.6 × g and was washed every 20 min during the equilibration time. Isometric tension was recorded with a force-displacement transducer (U-Gage, Shinkoh) connected to a carrier amplifier (AP-601G, Nihon Kohden) and a thermal pen recorder (WS-628G, Nihon Kohden).

After equilibration, the arterial rings were stimulated with phenylephrine (10⁻⁵ M). For experiments with antagonists or inhibitors, arterial rings were treated with the agents for 30 min prior to the stimulation, and phenylephrine (10⁻⁵ M) was applied in the presence of these agents. The effect of the absence of extracellular calcium ions was investigated by exposure of the arterial rings to a calcium-free solution for 10 min.

**Perfusion bioassay**

An arterial segment with intact endothelium (2–3 cm) was perfused intraluminally with oxygenated Krebs-Henseleit solution (37°C) at a constant flow of 2 ml/min using a roller pump (Harvard, 1210D). The perfusate emerging from the artery was dripped onto an endothelium-denuded arterial ring and the isometric tension was recorded as described above.

In a control experiment, the perfusate from a polyethylene tube, not through an endothelium-intact artery, was dripped onto a test ring.

**Data analysis and statistical processing**

Three parameters, i.e., the tension, amplitude, and frequency of the oscillations, were used to evaluate the effects of phenylephrine. The time course of changes in these parameters was monitored. The minimum point of each oscillatory contraction was measured for the tension. These parameters were expressed as the mean ± S.E.M. Significance of differences was tested using the Dunnett’s test.

**Drugs**

The following drugs were used: L-N-Methyl-monogarginine (L-NMMA), tetrodotoxin, guanethidine, atropine, indomethacin, diltiazem hydrochloride, cimetidine, propranolol hydrochloride, diphenhydramine hydrochloride, phenylephrine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), yohimbine hydrochloride, methylene blue and quercetin (Nacalai Tesque, Inc., Kyoto, Japan).

Calcium-free solution was prepared by omitting CaCl₂ from the Krebs-Henseleit solution and adding EGTA to a final concentration of 50 μM.

### RESULTS

**Characterization of phenylephrine-induced oscillation**

Rabbit mesenteric artery with intact endothelium displayed phasic contraction followed by tonic contraction superimposed with rhythmic oscillations when stimulated with phenylephrine (10⁻⁵ M) (Fig. 1). As the tension of the tonic contraction decreased, the amplitude of the oscillations gradually increased and reached a maximum when the tension reached its minimum level. Then the amplitude gradually decreased as the tonic contraction developed. The frequency of the oscillations changed in reverse order to the amplitude.

**Effects of adrenergic, cholinergic, and histamine antagonists as well as cyclooxygenase and lipoxygenase inhibitors**

The effects of adrenergic, cholinergic and histamine antagonists as well as cyclooxygenase and lipoxygenase inhibitors on the response to phenylephrine were examined. Yohimbine (10⁻⁶ M), propranolol (3 × 10⁻⁶ M), and atropine (10⁻⁶ M) were used to block α₂ and β-adrenergic and muscarinic cholinergic receptors, respectively. Guanethidine (10⁻⁶ M) and tetrodotoxin (3 × 10⁻⁷ M) were used to inhibit transmitter release from nerve terminals. Diphenhydramine (3 × 10⁻⁶ M) and cimetidine (10⁻⁷ M) were used to block histamine H₁ and H₂ receptors, respectively. Indomethacin (10⁻⁶ M) and quercetin (10⁻⁴ M) were respectively used to inhibit cyclooxygenase and lipoxygenase.

These antagonists or inhibitors had no effect on the oscillation induced by phenylephrine in arterial rings with intact endothelium.

**Effect of extracellular calcium**

The effects of diltiazem and the removal of extracellular calcium ions on the phenylephrine-induced responses of endothelium-intact arterial rings are shown in Fig. 2. Arterial rings treated with diltiazem (10⁻⁵ M) developed both phasic and tonic contractions when stimulated with phenylephrine and the initial phase of
the tonic contraction was increased. However, the amplitude and the frequency of the oscillations were significantly reduced as compared with the control preparations. In contrast, phenylephrine evoked only phasic contraction in the arterial rings exposed to calcium-free solution. Removal of extracellular calcium ions markedly attenuated the rhythmic oscillations.

Effects of the removal of endothelium, methylene blue and L-NMMA

The effects of the removal of the endothelium and the application of methylene blue and L-NMMA on the response to phenylephrine are shown in Fig. 3. The active tension induced by phenylephrine was potentiated by removal of the endothelium, whereas both the amplitude and the frequency of the oscillations were significantly suppressed.

In arterial rings with intact endothelium, methylene

![Graph](image1)

**Fig. 2.** Effects of diltiazem ($10^{-5}$ M) and the absence of extracellular calcium ions on the tension, amplitude, and frequency of the oscillation induced by phenylephrine ($10^{-5}$ M). Means ± S.E.M. of five animals are given for the control (○), for the arteries treated with diltiazem (□), and for the arteries subjected to the removal of extracellular calcium ions (▲). Asterisks (*) and **) indicate significant differences from the control at $P < 0.05$ and $P < 0.01$, respectively.

![Graph](image2)

**Fig. 3.** Effects of endothelium removal, methylene blue ($10^{-5}$ M) and L-N-methyl-endothelin (L-NMMA) ($3 \times 10^{-4}$ M) on the tension, amplitude and frequency of the oscillation induced by phenylephrine ($10^{-5}$ M). Means ± S.E.M. of five animals are given for the control (○), the endothelium-denuded arteries (□) and the arteries treated with methylene blue (▲) or L-NMMA (△). Asterisks (*) and **) indicate significant differences from the control at $P < 0.05$ and $P < 0.01$, respectively.
blue increased the resting tension. The phenylephrine-induced active tension was also potentiated by treatment with methylene blue \((10^{-5} \text{ M})\) or \(\text{L-NMMA} (3 \times 10^{-4} \text{ M})\). Methylene blue and \(\text{L-NMMA}\) both significantly reduced the amplitude and the frequency of the rhythmic oscillations. However, the inhibitory effect of \(\text{L-NMMA}\) was transient on the amplitude and incomplete on the frequency of the oscillations, respectively.

**Perfusion bioassay**

The perfusate containing phenylephrine \((10^{-5} \text{ M})\) from an endothelium-intact artery produced contraction and restored oscillations in an endothelium-denuded ring. The perfusate from the polyethylene tube also contracted the de-endothelialized test ring, but failed to induce the oscillation (Fig. 4).

![Fig. 4. Typical responses of an endothelium-denuded arterial ring to phenylephrine \((10^{-5} \text{ M})\) in the perfusion bioassay. Responses are induced by perfusate from the endothelium-intact artery (upper trace) or polyethylene tube (lower trace).](image)

**DISCUSSION**

Rabbit mesenteric arteries with intact endothelium displayed oscillatory responses when stimulated with phenylephrine. Since this oscillation was not affected by adrenergic, cholinergic and histamine antagonists as well as adrenergic presynaptic inhibitors, or by cyclooxygenase and lipoxygenase inhibitors, it seems unlikely that the release of nerve transmitter or the release of histamine, prostaglandins and/or leukotrienes was involved in the oscillation.

A dependence of the oscillation on extracellular calcium influx is evident from the inhibitory effect of the absence of extracellular calcium ions and the treatment with diltiazem. The release of endothelium-derived relaxing factor (EDRF) and contracting factor (EDCF) from endothelial cells is known to be calcium dependent; however, calcium antagonists have been shown not to prevent the release of either EDRF or EDCF (23). Thus it seems likely that activation of the voltage-dependent calcium channels of vascular smooth muscle cells may contribute to the oscillatory behaviors. It should be noted that diltiazem failed to inhibit both the phasic and tonic contractions induced by phenylephrine and increased the initial phase of the tonic contraction. The lack of inhibitory effect of diltiazem on both phasic and tonic contraction is consistent with the report that the calcium antagonist nifedipine inhibits the oscillatory response induced by norepinephrine more than it inhibits the phasic and tonic contraction of rabbit mesenteric arteries (20). Presumably, diltiazem has little effect on the calcium influx promoted by receptor activation at the concentration used in this study. However, we cannot explain why diltiazem increased the initial phase of the tonic contraction.

Methylene blue is known to be a non-specific inhibitor of soluble guanylate cyclase, the target enzyme of EDRF and nitro compounds (24), and \(\text{L-NMMA}\) is an inhibitor of the synthesis of nitric oxide, which has been found to be identical to EDRF (25). Based on the effects of removal of the endothelium and application of methylene blue or \(\text{L-NMMA}\), it appears that the rhythmic oscillation depends on the presence of the endothelium and possibly an endothelium-derived factor such as EDRF. However, the inhibitory effect of \(\text{L-NMMA}\) on the oscillation was incomplete, even though the concentration of \(\text{L-NMMA}\) used in this study has been shown to be effective (26). The reason for this observation is unclear. The finding that the perfusate emerging from the artery with intact endothelium restored the oscillation in the de-endothelialized arterial ring also suggests that an endothelium-derived factor mediates this rhythmic oscillation. Since EDRF can be released both spontaneously and by some agonists (13), the increase in phenylephrine-mediated contraction observed in endothelium-denuded rings or rings treated with methylene blue or \(\text{L-NMMA}\) may be explained by a lack of EDRF release. A rise of the resting tension by methylene blue could be due to inhibition of a background production of cGMP (27).

These rhythmic arterial responses may result from re-
petitive spikes of intracellular calcium ion concentration in vascular smooth muscle cells. Although the precise mediator or process responsible for the phenylephrine-induced tension oscillation could not be determined in the present study, a tempting hypothesis is that an endothelium-derived factor, such as EDRF and/or methylene blue-sensitive nitro compounds, activates the system regulating the intracellular calcium levels (including guanylate cyclase and the voltage-dependent calcium channel) which causes repetitive spikes of the intracellular calcium concentration in smooth muscle cells of rabbit mesenteric arteries.

In conclusion, the phenylephrine-induced oscillation in rabbit mesenteric arteries depends on the influx of extracellular calcium ions, presumably into vascular smooth muscle cells via the voltage-dependent calcium channels, and the presence of intact endothelium. The endothelium-derived factor therefore seems to act as the mediator of this oscillatory response.

REFERENCES