Flow-induced endothelium-dependent vasoreactivity in rat mesenteric arterial bed

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Abstract

We studied rat mesenteric arterial beds to determine the relationship between the effects of flow-induced shear stress and agonists on mesenteric vasoreactivity. When beds were perfused at gradually increasing flow rates, perfusion pressure was flow rate-dependently increased. The flow rate-mediated increase in perfusion pressure was significantly enhanced by Nω-nitro-L-arginine (L-NOARG) plus methylene blue (MB) and slightly enhanced by treatment with tetraethylammonium (TEA). In the presence of L-NOARG, MB, TEA, and indomethacin, the flow rate-induced increase in perfusion pressure was significantly enhanced, but this enhancement was significantly inhibited by combined treatment with BQ-123 plus BQ-788 (ETα and ETβ receptor antagonists, respectively). The ET-1 content of the perfusate was significantly increased following combined pretreatment with L-NOARG, MB, TEA, and indomethacin at a high flow rate. The methoxamine-induced contraction was significantly enhanced by NOS inhibition in both high- and low-flow-treated groups. The released nitrite level was significantly greater in high-flow-loaded than in the low-flow-loaded beds. We conclude that in this model, the response of vascular tone to flow stimulation is subtly regulated by endothelium-derived factors (especially, NO, endothelium-derived hyperpolarizing factor, and ET-1), and that these factors interact with each other.

Key words: contraction, endothelium, flow, mesenteric artery, shear stress

Introduction

Endothelial cells, which are situated at the interface between blood and the vessel wall, play a crucial role in controlling vascular tone through the release of a variety of factors that influence the contractile activity of the underlying smooth muscle (Cohen and Vanhoutte, 1995; Vanhoutte, 1998; Busse and Fleming, 2003). These relaxation factors include nitric oxide (NO) and prostacyclin, both diffusible factors (Moncada et al., 1976; Furchgott and Zadwadzki, 1980; Rapoport and Murad, 1983; Ignarro et al., 1987; Palmer et al., 1988). In addition, after blockade...
of NO and prostacyclin synthesis, stimulation of the endothelium is capable of evoking a vascular smooth muscle relaxation attributable to a third factor, endothelium-derived hyperpolarizing factor (EDHF) (Chen et al., 1988; Feletou and Vanhoutte, 1988; Garland et al., 1995; McGuire et al., 2001; Matsumoto et al., 2003). Endothelial cells also regulate vascular tone by releasing potent vasoconstrictors, such as endothelin-1 (ET-1) and thromboxane A₂ (Yanagisawa et al., 1988; Furchgott and Vanhoutte, 1989; Moreau, 1996; Buzzard et al., 1993). These regulatory functions of the endothelium can be modulated by endogenous substances (e.g., serotonin, bradykinin), pharmacological agents (e.g., acetylcholine, substance P), and mechanical forces (e.g., flow- or viscosity-mediated shear stress). Among these, shear stress is probably the most relevant physiological stimulus for the release of endothelial factors and thereby for the maintenance of normal vascular tone (Davies, 1995). In fact, previous studies in animal models have shown that shear stress stimulates the release of vasoactive factors by the microvascular endothelium (Stepp et al., 1999; Koller and Kaley, 1991; Koller et al., 1994).

Shear stress is determined by three variables: blood velocity, blood viscosity, and vascular diameter. When vessel diameter is reduced, or when blood velocity or viscosity is elevated, the shear stress imposed on the endothelium is increased (Hecker et al., 1993). In vivo studies have demonstrated that during the early phase of a flow increase, there is a period during which vascular diameter does not change; hence, the increase in flow is the result of an increase in flow velocity, and this consequently increases shear stress (Smiesko et al., 1989; Koller and Kaley, 1989; Koller and Kaley, 1990). This suggests that an increase in shear stress elicits a dilation that, in turn, will reduce shear stress, implying a causal relation between shear stress and dilation (Koller and Kaley, 1991). Vasodilation of resistance vessels occurs in response to increased perfusion flow so as to maintain tissue perfusion. The flow-induced vasodilation is mainly dependent on NO, which also regulates the vascular responsiveness to vasoconstrictors. In addition to NO, however, high flow increases the secretion of ET-1, a vasoconstrictor peptide, from endothelial cells. It is likely, therefore, that an interaction between NO and ET-1 plays a role, possibly a critical one, in the control of arterial vascular tone under conditions of high perfusion flow (Russo et al., 1999).

In view of the potential importance of flow-induced responses in mesenteric arterial beds, we designed this study to: 1) test whether flow-induced stimuli alter perfusion pressure; 2) test whether flow stimulates the release of a transferable substance from the endothelium, and 3) investigate whether flow-induced stimuli alter agonist-induced vasomotor activities. To ends, these rat mesenteric arterial beds were isolated, cannulated, and studied in vitro. We investigated the changes in perfusion pressure that occurred as flow rates were altered and also agonist-induced vasomotor activities in the presence and absence of flow stimuli. Furthermore, we quantified the mesenteric arterial beds transferable production of ET-1 and NOx (NO₂⁻ and NO₃⁻), nitric oxide metabolites at various levels of flow.
Methods

Materials

Bovine serum albumin (Fraction V), indomethacin, methoxamine hydrochloride, methylene blue, N\textsuperscript{6}-nitro-L-arginine (L-NOARG), papaverine hydrochloride, tetraethylammonium (TEA), and Triton X-100 were all purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetylcholine chloride (ACh) was from Daiichi Pharmaceuticals (Tokyo, Japan), while cyclo (D-\textrm{\alpha}-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (BQ-123) and N-[N-[N-\{(2,6-dimethyl-1-piperidinyl)carbonyl\}-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium (BQ-788) were from Research Biochemicals International (Natick, MA, U.S.A.). All drugs were dissolved in saline, except where otherwise noted. All concentrations are expressed as the final molar concentration of the base in the organ bath.

Animals

Male Wistar rats aged 18 weeks were housed under constant climatic conditions (temperature 21–22°C, relative air humidity 50 ± 5%). Food and water were allowed ad libitum to all animals. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Science, Sports, and Culture, Japan).

Preparation of the mesenteric arterial bed for perfusion

Rats anesthetized with diethyl ether were given an intravenous injection of 1,000 units/kg of heparin. Then, the mesenteric arterial bed was rapidly dissected out and placed into modified Krebs-Henseleit solution (KHS) containing 0.25% bovine serum albumin. This KHS solution consisted of (in mM): 118.0 NaCl, 4.7 KCl, 25.0 NaHCO\textsubscript{3}, 1.8 CaCl\textsubscript{2}, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 1.2 MgSO\textsubscript{4}, and 11.0 dextrose. The mesenteric artery and vein were tied off near the caecum, and the remaining intestine was separated from the arterial bed along the intestinal wall. The mesenteric arterial bed was then perfused using the method described previously by us (Kamata et al., 1996; Kamata and Makino, 1997; Makino and Kamata 1998). Briefly, warm (37°C), oxygenated (95% O\textsubscript{2}, 5% CO\textsubscript{2}) KHS was pumped into the mesenteric arterial bed, using a peristaltic pump operating at a rate of 5 ml/min, through a cannula inserted into the superior mesenteric artery. Vascular responses were detected as changes in perfusion pressure; this was monitored continuously by way of a pressure transducer (Nihon Kohden, Model AP2001, Tokyo, Japan) and recorded on a pen recorder. Following a 1 hr equilibration period, the perfusion circuit was transformed into a closed system by collecting the perfusate in a second bath and from thence recirculating it through the mesenteric arterial bed. The total volume of the closed system was 50 ml, and agents were administered via the bath. For the shear-stress study, the perfusion flow rate was increased in steps from 5 to 30 ml/min. When the perfusion pressure had reached a plateau at a given flow rate, the flow rate was increased to the next level (from 5 to 10, 20, and 30 ml/min). To investigate the influence of shear stress on agonist-induced vasoreactivity, concentration-response curves were constructed for the contraction induced by
methoxamine, and for the relaxation induced by ACh, after loading by exposure to a high flow rate (30 ml/min) for 2 hr. Dose-response curves for methoxamine ($10^{-7}$ ~ $3 \times 10^{-4}$ M) were obtained by cumulatively increasing the total concentration of the agonist in the bath. In the relaxation study, to standardize the vasodilator responses obtained with different drugs, papaverine ($10^{-4}$ M) was injected into each vascular bed and the resulting vasodilator response was expressed as 100%. In each preparation, once the methoxamine-induced contraction had reached a plateau (up to 50 mmHg), vasodilator responses to various concentrations of ACh ($10^{-10}$ ~ $10^{-5}$ M) were elicited in a cumulative manner. In some experiments, the mesenteric preparation was perfused with 0.05% Triton X-100 for 1 min to achieve functional removal of the endothelial cells lining the resistance vessels. This treatment reduced the ACh ($10^{-6}$ M)-induced vasodilation by more than 90% without reducing the contractile effects of methoxamine.

**Measurement of ET-1**

The perfusates were extracted using Amprep C2 columns (Amersham International plc.) following Amprep activation by 2 ml of 100% methanol followed by 2 ml of water. One milliliter of each sample was acidified with 0.25 ml 2 mol/l HCl, centrifuged at 10,000 g for 5 min at room temperature, then loaded onto the column. The column was then washed with 5 ml of 0.1% trifluoroacetic acid (TFA). Immunoreactive-endothelin was eluted with 2 ml of 80% acetonitrile/water containing 0.1% TFA. Then, the eluate was dried down under nitrogen, and the resulting pellet reconstituted using assay buffer (0.02 mol/l borate buffer, pH 7.4, containing 0.1% sodium azide). The concentration of ET-1 in the effluent was determined by radioimmunoassay using a commercially available kit (endothelin 1–21 specific [125I] assay system; Amersham International plc.).

**Measurement of NO$_2^-$ and NO$_3^-$**

We measured the content in the perfusate of NOx (NO$_2^-$ and NO$_3^-$). For this purpose, the perfusate was continuously collected on three occasions as follows; sample 1, after loading by exposure to 5 or 30 ml/min for 2 hr; sample 2, upon subsequent return to fresh KHS at 5 ml/min after methoxamine ($10^{-5}$ M) had been applied and the methoxamine-induced contractile response had stabilized (30 min), sample 3, after ACh ($10^{-6}$ M) had been applied and the ACh-induced relaxation response had stabilized (15 min). The concentration of nitrite and nitrate in the effluent from the mesenteric arterial beds was assayed by a method described elsewhere (Yamada and Nabeshima, 1997). Briefly, the NO$_2^-$ and NO$_3^-$ in each supernatant were separated by means of a reverse-phase separation column packed with polystyrene polymer (NO-PAK, 4.6 x 50 mm; EICOM, Kyoto, Japan), then NO$_3^-$ was reduced to NO$_2^-$ in a reduction column packed with copper-plated cadmium filings (NO-RED; EICOM, Kyoto, Japan). The NO$_2^-$ was mixed with a Griess reagent to form a purple azo dye in a reaction coil. The separation and reduction columns and the reaction coil were then placed in a column oven set at 35°C. The absorbance of the product dye at 540 nm was measured using a flow-through spectrophotometer (NOD-10, EICOM, Kyoto, Japan). The mobile phase, which was delivered by a pump at a rate of 0.33 ml/min, was 10% methanol containing 0.15 M NaCl/NH$_4$Cl and 0.5 g/L 4 Na-EDTA. The Griess reagent, which was 1.25% HCl containing 5 g/L sulfanilamide with 0.25 g/L N-
naphthylethylenediamine, was delivered at a rate of 0.1 ml/min. The concentrations of NO$_2^-$ and NO$_3^-$ and the reliability of the reduction column were examined in each experiment.

**Statistical analysis**

Data are expressed as the mean ± S.E. mean. When appropriate, statistical differences were assessed by means of Dunnett’s test for multiple comparisons after a one-way analysis of variance, a probability level of P<0.05 being regarded as significant. Statistical comparisons between concentration-response curves were made by means of a two-way ANOVA, with Bonferroni’s correction for multiple comparisons being performed post hoc (P<0.05 again being considered significant).

**Results**

*Effects of flow-induced changes in perfusion pressure on endothelium in perfused beds*

To investigate the effects of flow-induced shear stress on perfusion pressure in the mesenteric arterial bed, we performed a series of experiments in which flow-rate was raised step-wise in perfused beds. When flow was raised by steps (from 5 to 10, 20, and 30 ml/min), the perfusion pressure was flow-dependently increased (Fig. 1A). The flow-induced levels of perfusion pressure were significantly greater in endothelium-denuded than in endothelium-intact beds.

Since endothelial cells generate and release endothelium-derived factors, we investigated the effects of various inhibitors on the flow-dependent perfusion pressures in this bed. These experiments were carried out using endothelium-intact preparations (Fig. 1B). Combined treatment with L-NOARG (10$^{-4}$ M) plus MB (3 × 10$^{-5}$ M), a nitric oxide synthase (NOS) inhibitor and a soluble guanylate cyclase inhibitor, respectively, significantly enhanced the flow-dependent perfusion pressures, while TEA (10$^{-2}$ M), a non-selective K$^+$-channel blocker, produced only a slight, but nevertheless significant, enhancement. In contrast, indomethacin (10$^{-5}$ M), a cyclooxygenase inhibitor, had no effect.

Following treatment with a mixture of L-NOARG, MB, TEA and indomethacin, the flow-dependent perfusion pressures were greatly enhanced (Fig. 2). This enhancement was significantly inhibited by combined treatment with BQ-123 (3 × 10$^{-6}$ M) (an ET$_A$-receptor antagonist) plus BQ-788 (10$^{-6}$ M) (an ET$_B$-receptor antagonist), the pressure levels obtained being not different from those seen in endothelium-denuded preparations (Fig. 2).

*Change in release of ET-1 in flow-stimulated beds*

The basal concentration of ET-1 in the perfusate was not significantly different between the absence and presence of inhibitors (Table 1, 5 ml/min). When the beds were loaded by exposure to high flow rate (30 ml/min) for 2 hr, there tended to be a slightly increased ET-1 release. On the other hand, ET-1 release was significantly increased when beds were loaded by exposure to high flow in the presence of inhibitors (Table 1, 30 ml/min).
Effect of flow-induced shear stress on agonist-induced vasoreactivity in perfused beds

To investigate the influence of flow-induced shear stress on agonist-induced vasomotor activity, we performed experiments in which ACh (10^{-10} \text{ to } 10^{-5} \text{ M}) was added cumulatively to perfused beds precontracted by methoxamine in the absence or presence of L-NOARG after the beds had been loaded by exposure to a normal or high flow rate (5 or 30 ml/min) for 2 hr. The concentration-response curves for ACh shown in Fig. 3A reveal that the maximum vasodilation was not different among the groups. The high-flow-loaded L-NOARG-treated group exhibited a significantly lower sensitivity to ACh in the methoxamine-precontracted mesentery than the
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Untreated high-flow-loaded group, and a reduced sensitivity (vs. the relevant untreated control) was also shown by the TEA-treated normal- and high-flow-loaded groups (Fig. 3A, Table 2).

Similarly, as an agonist-induced contraction study, we performed experiments in which methoxamine \((10^{-7} \sim 3 \times 10^{-4} \text{ M})\) was added cumulatively to perfused beds in the absence or presence of L-NOARG after the beds had been loaded by exposure to a normal or high flow rate (5 or 30 ml/min) for 2 hr. The maximum response to methoxamine tended to be slightly reduced under high-flow-loading conditions (Fig. 3B, Table 2). Treatment with L-NOARG

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**Table 1** Flow-stimulated release of ET-1 as measured in the perfusate from mesenteric arterial beds

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>5</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh</td>
<td>0.080 ± 0.011</td>
<td>0.112 ± 0.015</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>0.073 ± 0.008</td>
<td>0.206 ± 0.020**###</td>
</tr>
</tbody>
</table>

Perfusate was collected from mesenteric arterial beds that were either untreated (Veh) or treated with a set of inhibitors [L-NOARG \((10^{-4} \text{ M})\), methylene blue \((3 \times 10^{-5} \text{ M})\), Indomethacin \((10^{-5} \text{ M})\), TEA \((10^{-5} \text{ M})\)] after loading at 5 or 30 ml/min for 2 hr. Each value represents the mean ± SEM from six experiments. **: P<0.01 vs. 30 ml/min veh-group. ###: P<0.001 vs. 5 ml/min inhibitors group.
greatly enhanced the sensitivity to methoxamine in both the normal- and high-flow-loaded groups (Fig. 3B, Table 2).

**Measurement of NO₂⁻ and NO₃⁻ in flow-stimulated beds**

The NO₂⁻ level in the perfusate from high-flow-loaded beds was significantly greater than at normal flow under both basal and agonist-stimulated conditions (Table 3). Furthermore, the NO₃⁻ level in the perfusate from high-flow-loaded beds was significantly increased (vs. normal
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Discussion

The major findings made in the present study concern the role of the endothelium in flow-induced stress and the regulation of perfusion pressure in the mesenteric arterial beds. Previous studies on many experimental models reported a flow-dependent vasodilation that is linked to an increased release of NO (Hecker et al., 1993; Joannides et al., 1995; Koller et al., 1994; Koller and Huang, 1994; 74: Pohl et al., 1991). In our system, flow-dependent perfusion pressure was significantly increased by removal of the endothelium, and by both L-NOARG plus MB and TEA in the endothelium-intact bed (Fig. 1). In this study, indomethacin had no

### Table 2

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Pretreatment</th>
<th>Flow rate</th>
<th>Max. response (%)</th>
<th>$-\log EC_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh</td>
<td>Flow</td>
<td>5</td>
<td>98.9 ± 0.43</td>
<td>8.14 ± 0.09</td>
</tr>
<tr>
<td>L-NOARG (10^{-4} M)</td>
<td>Flow</td>
<td>30</td>
<td>99.2 ± 0.65</td>
<td>8.09 ± 0.08</td>
</tr>
<tr>
<td>Indomethacin (10^{-5} M)</td>
<td>Flow</td>
<td>5</td>
<td>98.4 ± 0.98</td>
<td>8.00 ± 0.08</td>
</tr>
<tr>
<td>TEA (10^{-2} M)</td>
<td>Flow</td>
<td>30</td>
<td>97.5 ± 0.52</td>
<td>7.54 ± 0.09</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>NO$_2^-$ (nmol/min)</th>
<th>NO$_3^-$ (nmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh 5</td>
<td>0.167 ± 0.032</td>
<td>0.177 ± 0.011</td>
</tr>
<tr>
<td>30</td>
<td>0.683 ± 0.119*</td>
<td>0.275 ± 0.009***</td>
</tr>
<tr>
<td>Met 5</td>
<td>0.416 ± 0.118</td>
<td>0.179 ± 0.029</td>
</tr>
<tr>
<td>30</td>
<td>0.954 ± 0.231*</td>
<td>0.346 ± 0.027***</td>
</tr>
<tr>
<td>ACh 5</td>
<td>0.467 ± 0.120</td>
<td>0.174 ± 0.020</td>
</tr>
<tr>
<td>30</td>
<td>1.209 ± 0.260*</td>
<td>0.256 ± 0.086</td>
</tr>
</tbody>
</table>

Perfusate was collected from mesenteric arterial beds that were either untreated (Veh) or together with methoxamine (Met; 10^{-5} M) or ACh (10^{-6} M) after loading at 5 or 30 ml/min for 2 hr. Each value represents the mean ± SEM from five experiments. *: P<0.05, **: P<0.01, ***: P<0.001 vs. 5 ml/min group.
inhibitory effect on the flow-induced endothelium-dependent modification of perfusion pressure in the mesenteric arterial beds, indicating that vasodilator prostaglandins (PGs) may not play an important role in the relative relaxations seen when the endothelium was present. This is consistent with the previous finding that vasodilator PGs are not involved in shear stress-induced relaxation in the canine coronary artery (Holtz et al., 1984; Rubanyi et al., 1986). Our evidence suggesting that EDHF-mediated relaxation is involved in the flow-induced endothelium-dependent relaxation seen in our models is consistent with the recent report by Popp et al. (1998) that endothelial cells can liberate EDHF in response to shear stress, and with that by Takamura et al. (1999) that EDHF plays an important role in shear stress-induced endothelium-dependent relaxation, an effect in which K+ channels, especially calcium-activated K+ channels, appear to be involved. Thus, it is suggested that NO and EDHF play important roles as endothelium-derived factors in mediating decreases in perfusion pressure in this model.

We found that flow-induced levels of perfusion pressure were significantly attenuated by blockade of ET receptors under conditions involving inhibition of EDRF-mediated signaling (Fig. 2). Furthermore, ET-1 release was greatly increased by high-flow-loading under the same conditions (Table 1). To judge from previous reports, crosstalk between ET-1 and NO may play an important role in the control of vascular tone (Lavallee et al., 2001). Indeed, no only does NO impair ET-dependent effects, but it also limits stimulated ET-1 production, as shown by Boulanger et al. (Boulanger et al., 1990; Boulanger et al., 1991). Moreover, our data demonstrate that high-flow stimulation releases NO (Table 3; NO metabolites). Taken together, all this indicates that ET-1 release and its action may be constitutively inhibited by NO in the perfused mesenteric artery. Actually, ET-1 is released from both vascular smooth muscle cells (VSMC) and endothelial cells. Russo et al. (1999) suggested that in mesenteric beds, a high flow rate increases ET-1 production in VSMC. In intact arteries, although ET-1 release has been found to be abolished by phosphoramidon, an endothelin-converting-enzyme inhibitor, vascular responsiveness remained unchanged; however, vascular responsiveness was increased after endothelium removal, and this strongly supports a critical role for VSMC rather than for the endothelium (Russo et al., 1999). In addition, the release of ET-1 from arteries with an increased flow rate was similar in arteries with and without endothelium (Russo et al., 1999). In the study, following treatment with a mixture of L-NOARG, MB, TEA and indomethacin in endothelium-intact beds, flow-dependent perfusion pressure levels were greatly enhanced (Fig. 2). These enhanced perfusion pressures were significantly inhibited by treatment with a mixture of BQ-123 and BQ-788 (similar to endothelium-denuded levels) (Fig. 2). Hence, we suggest that in this model, ET-1 is released predominantly by endothelial cells and is an important factor mediating increases in perfusion pressure (contractile effect).

To examine the possible physiologic importance of flow stimuli in agonist-induced vasoreactivity, we performed experiments in which ACh (muscarinic agonist) and methoxamine (α1-agonist) were added cumulatively to perfused mesenteric arterial beds after loading at a high or low flow rate for 2 hr. As shown in Fig. 3, the ACh-induced vasodilation showed no significant alteration between high- and low-flow-loading, nor was the ACh-induced vasodilation significantly different between L-NOARG-treated and -untreated beds at low flow. However, the ACh-induced vasodilation was significantly weaker in the presence of L-NOARG than in its
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absence after high-flow loading (Fig. 3, Table 2). These results suggest that NO makes an important contribution to ACh-induced relaxation in the flow-stimulated perfused mesenteric artery. However, there was no significant difference in ACh-induced vasorelaxation between low- and high-flow stimulation. It is therefore suggested that muscarinic receptor affinity and efficacy are not different between the absence and presence of flow stimulation. Further investigation will be required on this point. The ACh-induced vasodilation was significantly weaker in the presence of TEA than in its absence in low- and high-flow-loaded mesenteric arteries. In our previous report, we felt that the vasodilator response of the mesenteric arterial bed to ACh might be attributable to NO and EDHFs (Makino et al., 2000). In other reports, the importance of EDHF in endothelium-dependent relaxation in the rat mesenteric circulation was said to increase as the vessel size decreased (Shimokawa et al., 1996), and EDHF was said to play an important role in shear stress-induced endothelium-dependent relaxation (Takamura et al., 1999). Taking all this together leads us to speculate that ACh-induced EDHF release is at a similar level between low- and high-flow conditions. When shear stress is induced in the mesenteric arterial bed, EDHF signaling increases (see above). However, once flow-induced shear stress was stopped, EDHF signaling returned to the basal level and exerted no influence over ACh-induced vasorelaxation. These results suggest that the endothelium plays an important role in determining the magnitude of the ACh-induced vasodilator response in flow-stimulated mesenteric arterial beds (i.e., both NO and EDHF can modulate this agonist-induced vasodilator response).

The vasoconstriction induced in the mesenteric arterial bed by methoxamine, an α1-adrenoceptor agonist, is influenced by endothelium-derived factors after flow stimulation. In our previous study, the mesenteric vasoconstriction induced by methoxamine in control rats was markedly inhibited by BQ-123 + BQ-788, and methoxamine caused a slight release of ET-1 (Makino et al., 1998). In the present study, the methoxamine-induced vasoconstriction tended to be decreased in high-flow-loaded beds (Fig. 3B, Table 2), but showed no significant alteration between the presence and absence of indomethacin or TEA in both low- and high-flow-loaded beds (Table 2). This suggests that PGs and EDHF may not be involved in the methoxamine-induced vasoconstriction after high-flow stimulation. On the other hand, the methoxamine-induced vasoconstriction was markedly enhanced by treatment with L-NOARG in both high- and low-flow-loaded beds (Fig. 3B, Table 2), indicating that methoxamine releases NO from the endothelium, as attested by the data in Table 3 and by our previous report that methoxamine releases NO from the endothelium of the rat mesenteric arterial bed (Kamata and Makino, 1997). Moreover, there was significant NO release in control and methoxamine-treated beds after high-flow stimulation (Table 3). As mentioned above, NO impairs endothelin production/action in vitro (Lavallee et al., 2001). These results suggest that the endothelium may play an important role in determining the magnitude of the methoxamine-induced vasoconstrictor response in the flow-stimulated mesenteric arterial bed (i.e., both NO and ET-1 can modulate this agonist-induced vasoconstrictor response).

Unexpectedly, the methoxamine-induced vasoconstriction seen in the presence of L-NOARG was significantly weaker in high-flow-loaded beds than low-flow-loaded ones (Fig. 3B). It was previously reported that the second dose-response curve obtained for the methoxamine-induced
increase in perfusion pressure in the mesenteric arterial bed shows is significantly weaker than the first, and that this decrease in the response is significantly inhibited by removal of the endothelium or by pretreatment with L-NOARG (Kamata and Makino, 1997). Thus, the authors of that paper suggested that a desensitization of the methoxamine-induced contraction develops rapidly in the mesenteric arterial bed, and that release of NO from the endothelium plays a major role in this desensitization (Kamata and Makino, 1997). We speculate that the diminished methoxamine-induced contractile response seen in the presence of L-NOARG may be attributable to a desensitization via increased NO release in the high-flow-loaded mesenteric arterial bed.

Taken together, the above evidence suggests that a critical association exists between flow rate and the release of vasoactive substances, and that these factors play a vital role in the regulation of vascular responsiveness in the mesenteric arterial bed.

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