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Mechanisms underlying endothelium-dependent flow increase in perfused rat mesenteric vascular bed

Hideyuki Fujioka, Kazuhide Ayajiki, Kazuya Shinozaki, Tomio Okamura*

Department of Pharmacology, Shiga University of Medical Science, Seta, Otsu, Shiga 520-2192, Japan

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Abstract

The isolated rat mesenteric vasculature was perfused at constant pressures of 40, 80 or 120 mm Hg and the change in flow rate was measured. In the presence of phenylephrine, treatment with 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS) or N^G-nitro-L-arginine (L-NA) significantly inhibited the pressure-dependent flow rate increase, but treatment with indomethacin or charybdotoxin plus apamin did not. Acetylcholine, bradykinin and ADP increased the flow rate, which had been markedly suppressed by CHAPS. At 80 mm Hg, the flow rate increase induced by these agonists was not affected by indomethacin plus L-NA, but was suppressed by subsequent treatment with charybdotoxin plus apamin. Changes in the perfusion pressure did not significantly affect the flow rate increases induced by the agonists. In conclusion, the opening of charybdotoxin plus apamin-sensitive Ca^{2+}-dependent K^+ channels may be mainly involved in the endothelium-dependent flow rate increase induced by the agonists, whereas nitric oxide (NO) may be responsible for the endothelium-dependent, pressure-induced flow rate increase.

Keywords: Constant-pressure perfusion; Mesenteric vascular bed; (Rat); Endothelium; NO (Nitric oxide); K^+ channel, Ca^{2+}-dependent

1. Introduction

Vascular endothelium plays important roles in the regulation of vascular tone. Endothelium-dependent relaxation is mediated by nitric oxide (NO) (Moncada et al., 1991), vasodilator prostanoids and/or endothelium-derived hyperpolarizing factor (EDHF) which hyperpolarizes cell membranes and induces smooth muscle relaxation (Vanhoutte and Mombouli, 1996). It has been reported that it is not only chemical stimulation induced by vasoactive agents such as acetylcholine and bradykinin but so does mechanical stimulation such as shear stress produced by blood flow that causes endothelium-dependent vasodilation (Busse and Fleming, 2003). Many researchers have investigated the mechanisms underlying such endothelium-dependent vasodilation in various arteries and arterioles from different animals, and as a result, EDHF is thought to play a more major role in endothelium-dependent relaxation in the arterioles and microcirculation than NO (Busse et al., 2002; Hwa et al., 1994; Takamura et al., 1999). However, there is still only limited information about the function of physiological regulators derived from vascular endothelium in maintaining tissue blood flow in response to hemodynamic changes. To find out more about this, different types of studies have been performed on perfused vasculatures. Randall and Hiley (1988) investigated the pressure–flow relationship with a constant–flow perfusion system and similar systems have been used to evaluate the endothelium-dependent vasodilation induced by agonists (Fulton and Quilley, 1998; Moore et al., 1990). Further, constant-pressure perfusion systems have been used to examine the effect of shear stress on endothelial function by measuring the diameter of arterial segments in the rat (Sun et al., 2001; Takamura et al., 1999) and rabbit (Cooke et al., 1991). However, very few studies have measured flow rate at a constant pressure in order to examine the endothelial control of vasodilation. Therefore, for the present study, we developed a constant-pressure vascular perfusion system with the aim of investigating agonist-induced endothelium-dependent flow increases and the effect of changes in perfusion-pressure on the endothelial control of vascular tone in the mesenteric circulation of rats.

* Corresponding author. Tel.: +81-77-548-2181; fax: +81-77-548-2183.
E-mail address: okamura@belle.shiga-med.ac.jp (T. Okamura).

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2. Materials and methods

The Animal Care and Use Committee at Shiga University of Medical Science approved the use of rat mesenteries in accordance with the experimental protocols of this study.

2.1. Preparations

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 300–400 g were given 2000 U/kg heparin intravenously and then killed by bleeding from the common carotid arteries under deep general anesthesia with ether. The superior mesenteric artery was cannulated, and the mesentery was isolated together with the intestine. All branches of the superior mesenteric artery other than those irrigating the intestine were ligated. A hole was made in the main trunk of the mesenteric vein for the outflow. The mesenteric artery was continuously perfused with Krebs-Henseleit solution at a constant pressure of 80 mm Hg achieved by adjusting the height of the reservoir containing the perfusion solution. The perfusion pressure was monitored with a pressure transducer (MP5100, Baxter, Tokyo, Japan) connected to a pressure monitoring system (AP641G, Nihon Kohden, Tokyo, Japan). In some experiments, the perfusion pressure was adjusted to 40 or 120 mm Hg by changing the height of the reservoir (Fig. 1). Constituents of the Krebs-Henseleit solution were (in mM) 120 NaCl, 5.4 KCl, 2.2 $CaCl_2$, 1.0 $MgCl_2$, 25.0 $NaHCO_3$, and 5.6 glucose and the pH was 7.36–7.43. The solution was maintained at 37.0 ± 0.5 °C and aerated with a mixture of 95% O_2–5% CO_2. The isolated tissue was placed on a grid and its exterior was kept wet by dripping the solution (0.5 ml/min) on to it. The flow rate of the perfusate was recorded by an electromagnetic flowmeter (NFV-2100, Nihon Kohden) whose probe was hooked on to the main trunk of the mesenteric artery. The drugs were applied directly into the reservoir containing the perfusion solution.

2.2. Pressure-induced response

In the experiments regarding the pressure-induced response of the preparation, an equilibration period of 30 min at 40 mm Hg was used. In order to obtain the pressure-induced response, the perfusion pressure was changed to 80 and 120 mm Hg, and then returned to 40 mm Hg by changing the height of the reservoir. In some preparations, a solution containing 0.4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS) was perfused for 30 s to remove the endothelium (Moore et al., 1990), followed by washout with the drug-free solution. The effect of CHAPS was confirmed by the absence of a flow increase due to 10^{-6} M acetylcholine and the presence of a response to 10^{-6} M sodium nitroprusside before the experiments were started. The pressure-induced response of non-treated (control) and CHAPS-treated preparations was compared in parallel. For experiments on the effect of the blocking agents(s) on the pressure-induced response, the agent(s)

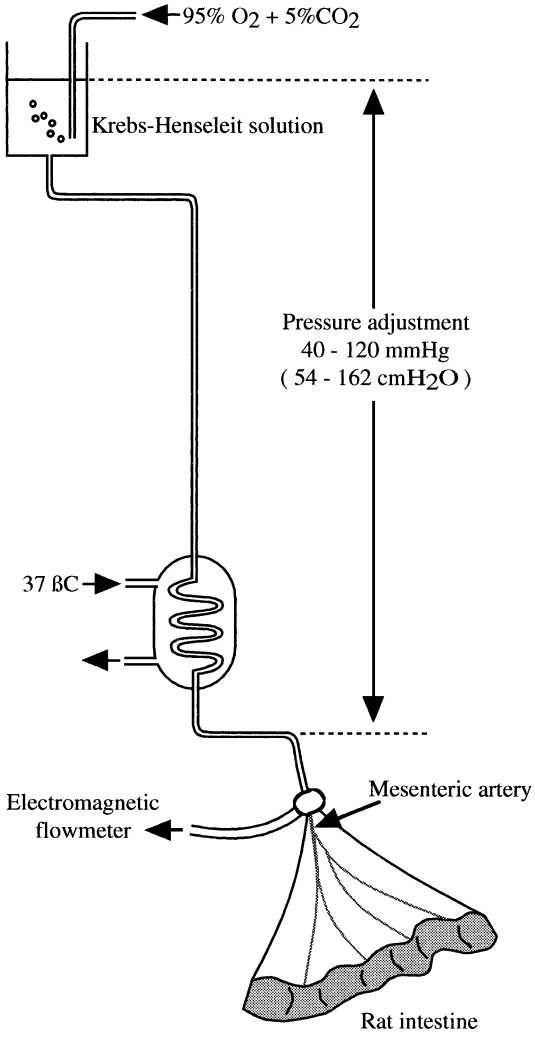

Fig. 1. Schema of constant-pressure vascular perfusion system.

(indomethacin, N^G-nitro-L-arginine (L-NA), charybdotoxin plus apamin) were added successively to the same preparations at the perfusion pressure of 40 mm Hg. After one pressure-induced response had been obtained without treatment (control), the blocking agent(s) was added, and then the next pressure-induced response was obtained after the equilibration of the blocking agent(s) for 30 min in the absence or presence of phenylephrine. In order to examine the pressure-induced response under conditions of slight vasoconstriction, phenylephrine ($[1.0–2.0] \times 10^{-5}$ M) was added to achieve a 20–30% decrease in flow at 40 mm Hg. The concentration of phenylephrine used was the same in each preparation. Therefore, the concentrations of phenylephrine were not variable within and between groups in the experiment testing the pressure-induced response.

2.3. Agonist-induced response

In the experiments on the agonist-induced response, the initial dose–response curves for acetylcholine, bradykinin, ADP and sodium nitroprusside were compared in different

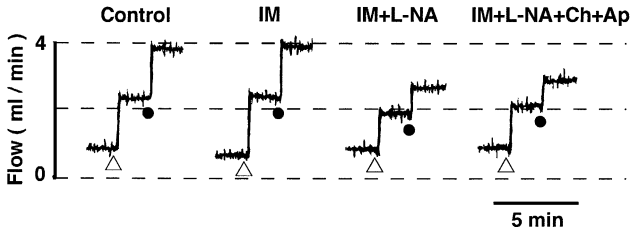

Fig. 2. Recordings of the pressure-dependent flow changes in the presence and absence of indomethacin, N^G-nitro-L-arginine (L-NA), and charybdotoxin plus apamin in the perfused rat mesenteric vascular bed. Phenylephrine (10^{-5} M) was administered continuously. These blocking agents were applied successively to the respective preparation. Control: no treatment, IM: treatment with indomethacin (10^{-6} M), IM + L-NA: additional treatment with L-NA (10^{-4} M), IM + L-NA + Ch + Ap: additional treatment with charybdotoxin (10^{-7} M) and apamin (10^{-6} M). Change in perfusion pressure: Δ, from 40 to 80 mm Hg; ●, from 80 to 120 mm Hg.

mesentery–intestine preparations pretreated with phenylephrine at 80 or 120 mm Hg, since tachyphylaxis was developed by repeated applications of these substances. These experiments were not performed at the low perfusion pressure of 40 mm Hg, because the flow rate at this pressure was too low to obtain reliable measurements. The changes in flow rate induced by the vasoactive substances were expressed relative to the change induced by withdrawal of phenylephrine (100%). In some experiments, the effect of CHAPS on the agonist-induced response was examined. Effects of blocking agents (indomethacin, L-NA, charybdotoxin plus apamin) on the flow rate changed by the agonists were examined at 80 mm Hg. In the preliminary study, the effect of phenylephrine at the same concentration on the flow rate differed in the presence and absence of blocking agents. Therefore, different concentrations of phenylephrine were added to obtain the similar extent of the decrease in the flow rate (around 1.5 ml/min) regardless of the presence of blocking agent(s); the mean values ± S.E. of the concentrations of phenylephrine were $[56 \pm 12.6] \times 10^{-6}$ M ($n = 10$, control), $[1.5 \pm 0.2] \times 10^{-6}$ M ($n = 10$, treated with CHAPS), $[7.7 \pm 1.0] \times 10^{-6}$ M ($n = 10$, treated with 10^{-4} M of L-NA and 10^{-6} M of indomethacin) and $[1.5 \pm 0.2] \times 10^{-6}$ M ($n = 10$, treated with 10^{-4} M of L-NA, 10^{-6} M of indomethacin and 10^{-7} M of charybdotoxin plus 10^{-6} M of apamin).

2.4. Statistics

The results shown in the text and figures are expressed as mean values ± S. E. Statistical analyses were made using Student's unpaired *t*-test for two groups and Tukey's test after one-way analysis of variance (ANOVA) for three or more groups.

2.5. Drugs used

Drugs used were N^G-nitro-L-arginine (L-NA), charybdotoxin, apamin, bradykinin (Peptide Institute, Minoh, Japan), acetylcholine chloride (Daiichi Pharmaceutical, Tokyo, Japan), sodium nitroprusside (Merck, Darmstadt, Germany), indomethacin, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS), L-phenylephrine (Sigma, St. Louis, MO), and adenosine-5′-diphosphate disodium (ADP; Nacalai Tesque, Kyoto, Japan). The drugs, except indomethacin, were dissolved in water as a stock solution. Indomethacin was dissolved in bicarbonate buffer (pH = 9.2). The drugs were added directly to the perfusate, and the final concentrations of the drugs were 1/100 or 1/1000 of the stock solutions. No vehicle effect was observed.

3. Results

3.1. Pressure-induced response

Increases in the perfusion pressure in the rat mesenteric vascular beds produced pressure-dependent increases in the flow rate in the absence of phenylephrine. The absolute flow rates at 40, 80 and 120 mm Hg were 1.2 ± 0.1, 2.9 ± 0.2, and 4.5 ± 0.3 ml/min, respectively ($n = 12$). The pressure–flow relationship was maintained throughout the experiment (at least for 3 h). In the absence of phenylephrine, the pressure-dependent increase in flow rate was not significantly affected by treatment with CHAPS, indomethacin, L-NA, charybdotoxin or apamin ($n = 6$; data not shown).

Phenylephrine (10^{-5}–2×10^{-5} M) was added until a 20–30% decrease in flow rate was obtained at 40 mm Hg

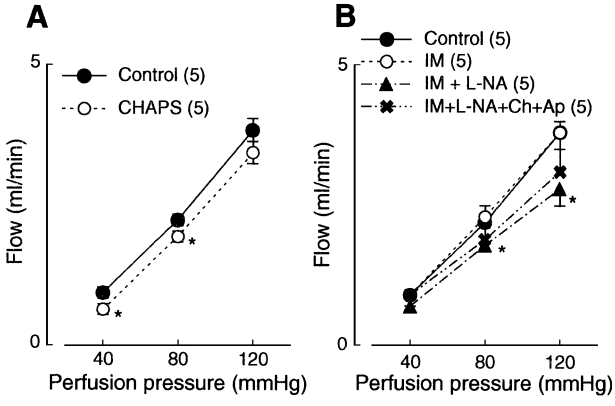

Fig. 3. Pressure–flow relationships in the presence and absence of CHAPS (panel A); indomethacin, L-NA and charybdotoxin plus apamin (B), in the perfused rat mesenteric vascular bed. Phenylephrine (10^{-5}–2×10^{-5} M) was continuously administered (A) Pressure–flow relationships for preparations with and without CHAPS. Control: no treatment, CHAPS: treatment with 0.4% CHAPS for 30 s. (B) The blocking agent(s) were applied successively to the respective preparation. Control: no treatment, IM: treatment with indomethacin (10^{-6} M), IM + L-NA: additional treatment with N^G-nitro-L-arginine (10^{-4} M), IM + L-NA + Ch + Ap: additional treatment with charybdotoxin (10^{-7} M) and apamin (10^{-6} M). Numbers in parentheses indicate the number of preparations. *Significantly different from control: $P < 0.05$. Vertical bars represent S.E.

in the experiment on the pressure-induced response. Under these conditions, the addition of blockers (indomethacin, L-NA, charybdotoxin and apamin) did not decrease the perfusion flow rate. The concentration of phenylephrine was fixed at the following conditions with 80 and 120 mm Hg in the same preparations. The absolute flow rates at 40, 80, and 120 mm Hg in the presence of phenylephrine were 0.9 ± 0.1 ($P>0.05$, compared with the values without phenylephrine, unpaired t-test), 2.2 ± 0.1 ($P<0.05$), and 3.8 ± 0.2 ($P>0.05$) ml/min, respectively ($n=5$). While treatment with indomethacin did not affect the pressure-dependent flow increase in this case, the addition of L-NA significantly reduced it at 80 and 120 mm Hg. When charybdotoxin and apamin were then added, the pressure-dependent flow increase was not reduced further as shown in Fig. 2. Treatment with CHAPS, however, significantly reduced the increase in the flow rate. The data are summarized in Fig. 3. Inhibition by L-NA was consistently observed even when the sequence of addition of the inhibitors was randomized, and was not in the order described above.

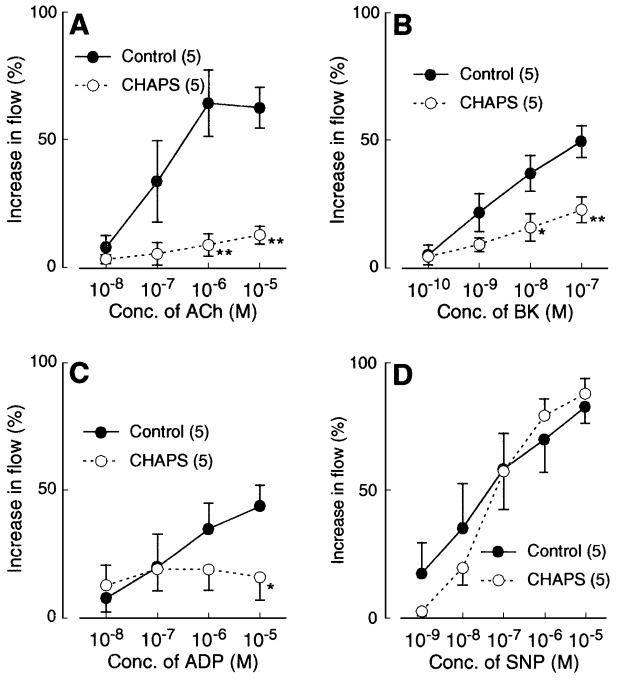

Fig. 4. Effect of treatment with 0.4% CHAPS for 30 s on the flow increase induced by acetylcholine (ACh, panel A), bradykinin (BK, panel B), ADP (panel C) and sodium nitroprusside (SNP, panel D) in the perfused rat mesenteric vascular bed pretreated with phenylephrine. The perfusion pressure was 80 mm Hg. CHAPS was added before application of the agonists and only initial responses to the agonists were compared between preparations with and without CHAPS. Phenylephrine (3×10^{-5}–7×10^{-5} M for control preparation and 10^{-6}–3×10^{-6} M for preparation after treatment with CHAPS, respectively) was administered. The ordinate denotes the flow increase relative to that induced by withdrawal of phenylephrine (100%). Numbers in parentheses indicate the number of preparations used. *Significantly different from control: $P<0.05$, $^{**}P<0.01$. Vertical bars represent S.E.

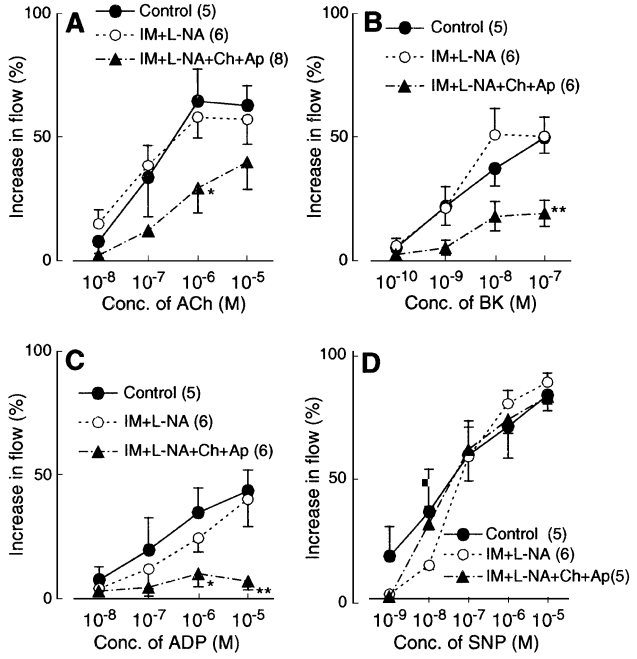

Fig. 5. Effect of treatment with indomethacin (10^{-6} M, IM) plus N^{G}-nitro-L-arginine (10^{-4} M, L-NA) and IM, L-NA and charybdotoxin (10^{-7} M, Ch) plus apamin (10^{-6} M, Ap) on the flow increase induced by acetylcholine (ACh, panel A), bradykinin (BK, panel B), ADP (panel C) and sodium nitroprusside (SNP, panel D) in the perfused rat mesenteric vascular bed pretreated with phenylephrine. The perfusion pressure was 80 mm Hg. The blocking agents were applied to the respective preparations before the agonists and only initial responses to the agonists were compared between preparations with and without blocking agents. Phenylephrine (3×10^{-5}–7×10^{-5} M for control preparation, 5×10^{-6}–10^{-5} M for preparation treated with indomethacin plus L-NA and 10^{-6}–3×10^{-6} M for preparation treated with indomethacin, L-NA and charybdotoxin plus apamin, respectively) was added. The ordinate denotes the flow increase relative to that induced by withdrawal of phenylephrine (100%). Numbers in parentheses indicate the number of preparations used. *Significantly different from control: $^{*}P<0.05$, $^{**}P<0.01$. Vertical bars represent S.E.

3.2. Agonist-induced response

In the presence of phenylephrine, infusion of acetylcholine, bradykinin and ADP dose dependently increased the flow rate in the perfused rat mesenteric vascular beds at 80 mm Hg. The flow rate increases were significantly reduced in the preparations treated with CHAPS (Fig. 4A–C). However, treatment with CHAPS did not reduce the flow increase induced by sodium nitroprusside (Fig. 4D). The flow rate increases induced by acetylcholine, bradykinin and ADP were not affected in the preparations treated with indomethacin plus L-NA, but were reduced in the preparations treated with indomethacin, L-NA and charybdotoxin plus apamin (Fig. 5A–C). The flow rate increase induced by sodium nitroprusside, however, was not affected in the preparations treated with indomethacin, L-NA and charybdotoxin plus apamin (Fig. 5D).

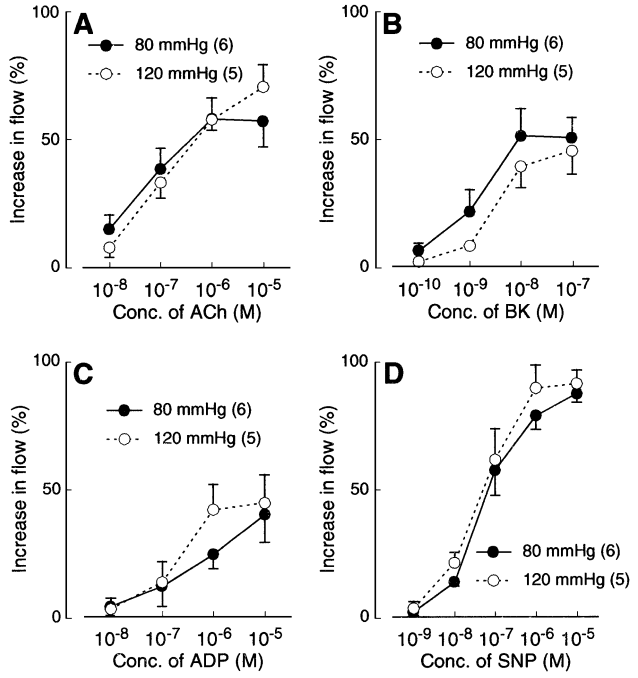

Fig. 6. Effect of changing perfusion pressure from 80 to 120 mm Hg on the flow increase induced by acetylcholine (ACh, panel A), bradykinin (BK, panel B), ADP (panel C) and sodium nitroprusside (SNP, panel D) in the perfused rat mesenteric vascular bed pretreated with phenylephrine. The pressure was adjusted to 80 or 120 mm Hg, and the initial responses to the agonists were compared among different preparations. Phenylephrine (3×10^{-5}–7×10^{-5} M at 80 and 120 mm Hg) was administered. The ordinate denotes the flow increase relative to that induced by withdrawal of phenylephrine (100%). Numbers in parentheses indicate the number of preparations used. Vertical bars represent S.E.

The flow rate increases induced by acetylcholine, bradykinin, ADP and sodium nitroprusside at 80 and 120 mm Hg did not differ significantly (Fig. 6).

4. Discussion

In the present study, we used a new constant-pressure perfusion system to perfuse the isolated whole rat mesentery attached to the intestine in order to investigate the role of endothelium in the regulation of mesenteric circulation. In the range of 40–120 mm Hg, the flow rate was dependent on the pressure. In the absence of phenylephrine, pressure-dependent flow rate increases were not affected by treatment with CHAPS, a detergent which suppresses endothelial function (Moore et al., 1990), indomethacin, a cyclooxygenase inhibitor, L-NA, a nitric oxide synthase inhibitor (Moore et al., 1990), or charybdotoxin plus apamin, two Ca^{2+}-dependent K^+ channel blockers (Garcia et al., 1991). This suggests that the pressure-dependent flow increase under these experimental conditions was not due to vasodilating prostanoids, NO nor to the opening of Ca^{2+}-dependent K^+ channels sensitive to charybdotoxin plus apamin. However, when vascular resistance had been increased by application of phenylephrine, the pressure-induced flow increase was significantly inhibited by treatment with CHAPS and L-NA, but was not affected by treatment with indomethacin or charybdotoxin plus apamin. These findings suggest that endothelium is slightly but significantly involved in the control of the flow rate in response to mechanical forces. Endothelium-derived NO, but not prostanoids or substance(s) opening Ca^{2+}-dependent K^+ channels sensitive to charybdotoxin plus apamin, is mainly involved in the control of the flow rate under these conditions.

The release of NO by phenylephrine is probably due to mechanical forces rather than to the α_1-adrenoceptor stimulation. Because the concentrations of phenylephrine used for the experiment on the pressure-induced response were the same in the absence and presence of blocking agent(s), the amount of NO released by phenylephrine seems to be the same. However, the inhibitory effect of L-NA at 120 mm Hg was more potent than that at 80 mm Hg, therefore, involvement of NO released by α_1-adrenoceptor stimulation, if any, in this response seems to be minimal. These results also imply that an endothelial response to mechanical stimulation may not be observed in arteries in the absence of vasoconstricting influences, such as sympathetic nerve action and vasoconstricting agents. Thus, tonic vasoconstriction elicited by the infusion of phenylephrine enabled us to observe an endothelial response and brought our system closer into line with physiological conditions.

Previous studies have indicated that mechanical forces such as shear stress activate endothelial K^+ channels (Jacobs et al., 1995; Olesen et al., 1988; Rusko et al., 1992), and induce the release of NO (Cooke et al., 1991; Ohno et al., 1993). Also, a decrease in the radius of the conduit artery perfused at a constant flow rate was seen to activate NO production (Ayajiki et al., 1996). These findings suggest that an increase in shear stress activates endothelial NO synthase and stimulates the release of NO from the endothelium. Miura et al. (2001) reported that both NO, which opens charybdotoxin-sensitive Ca^{2+}-activated K^+ channels, and NO were involved in the flow-induced dilation of human coronary arterioles precontracted by endothelin-1. Further, Takamura et al. (1999) reported that EDHF plays an important role in shear stress-induced relaxation in rat mesenteric arteries precontracted by phenylephrine. In contrast, our results suggest that NO is more important than the opening of Ca^{2+}-dependent K^+ channels sensitive to charybdotoxin plus apamin in the regulation of the pressure-dependent flow increase. These differences may be due to the fact that rat mesenteric vasculature preparations, perfused mesenteric arterial segments, were used rather than whole mesentery. The reason for the difference in the involvement of EDHF in the pressure-dependent flow increase in human coronary arterioles and perfused rat mesenteric vascular beds in the present study is not known. It may be due to species differences or to the location of the blood vessels used in the experiment.

As mentioned above, we found that increases in the flow rate induced by acetylcholine, bradykinin and ADP were inhibited by CHAPS and charybdotoxin plus apamin, but were not affected by L-NA or indomethacin. Also, the sodium nitroprusside-induced flow increase was not affected by CHAPS or charybdotoxin plus apamin. Thus, the agonist-induced vasodilation is thought to be endothelium-dependent, and to be mediated by the opening of Ca^{2+}-dependent K^+ channels sensitive to charybdotoxin plus apamin, but not by the release of NO or vasodilating prostanoids. EDHF produced in rat mesenteric arteries has been reported to be sensitive to charybdotoxin plus apamin (Chen and Cheung, 1997), and several candidates for EDHF have been reported (McGuire et al., 2001). Bolotina et al. (1994) reported that NO can activate charybdotoxin-sensitive Ca^{2+}-activated K^+ channels in rat vascular smooth muscle, and Weidelt et al. (1997) reported that sodium nitroprusside may open both ATP-dependent and Ca^{2+}-activated K^+ channels in rat mesenteric arteries.

One may suppose that the agonists used could stimulate the release of NO from the endothelium of the proximal part of rat mesenteric artery (Fukuda et al., 1992; Kähönen et al., 1995) and that the released NO could dilate the distal part of the artery via activation of K^+ channels. However, this was not the case in the present study, since charybdotoxin plus apamin inhibited the agonist-induced flow increase, whereas L-NA did not, and the sodium nitroprusside-induced flow increase was not suppressed by charybdotoxin plus apamin. Cytochrome *P*450 (CYP) products are considered to be EDHF in monkey lingual (Ayajiki et al., 1999) and porcine coronary arteries (Fisslthaler et al., 1999), since endothelium-dependent relaxation mediated via charybdotoxin plus apamin-sensitive Ca^{2+}-activated K^+ channel opening was inhibited by CYP inhibitors. Gap junctional communication between vascular endothelium and smooth muscle may play an important role in the endothelium-dependent and L-NA methyl ester (L-NAME)-resistant relaxation of rat arteries (Chaytor et al., 2000; Taylor et al., 1998). H_2O_2 is also reported to be a diffusible candidate for EDHF in human mesenteric arteries (Matoba et al., 2002). Further investigations are required to identify the EDHF in rat mesenteric vascular bed.

The flow increase induced by the agonists was not affected by changing the perfusion pressure in the present study. This suggests that the synthesis and release of substance(s) opening Ca^{2+}-dependent K^+ channels sensitive to charybdotoxin plus apamin induced by the agonists would not be affected by the change in blood pressure. No other information has so far been found about the acute effect of changing the pressure on the EDHF-related flow increase stimulated by agonists in the rat. The involvement of EDHF in the endothelium-dependent vasodilation, however, has been compared between normotensive and hypertensive rats. On treatment with NO synthase inhibitors, the vasodilation induced by acetylcholine in isolated distal mesenteric artery segments was suppressed in spontaneously hypertensive rats as compared with Wistar-Kyoto rats (Izzard and Heagerty, 1999). Since the acetylcholine-induced vasodilation was endothelium-dependent in this case, the action of EDHF stimulated by this agonist appears to be suppressed in spontaneously hypertensive rats. However, this may not be the case for other types of hypertension. Sofola et al. (2002) reported that the hypertension induced by a high-salt diet for 4 weeks did not inhibit acetylcholine-induced dilation of perfused rat mesenteric arteries treated with L-NAME and indomethacin, as compared with normotensive rats. Further, Randall and March (1998) demonstrated that endothelium-dependent vasodilation resistant to L-NAME and indomethacin in perfused mesenteric arteries from transgenic hypertensive (Renin-overexpressed) rats was not suppressed, as compared with normotensive rats. Therefore, in the rat mesenteric vascular bed, a state in which blood pressure is already high or is suddenly elevated may not itself affect the agonist-stimulated synthesis/release of substance(s) opening Ca^{2+}-dependent K^+ channels sensitive to charybdotoxin plus apamin.

There are many useful parameters for evaluating the circulatory system. Under in vivo conditions, blood flow to the tissue is one of the important factors regarding the local circulation, whereas the flow rate has mostly not been measured under in vitro conditions, possibly because of the technical difficulty. The data obtained from this system can more directly reflect the change in the flow rate than do other methods, i.e. measurement of change in the pressure or in the diameter of the arterial or arteriolar segment. This system allows us to examine the effects of drugs under different pressure conditions. Further, the role of the endothelium can also be evaluated in this system. There are very few studies that measured flow rate at constant pressure so as to examine the endothelial control of vasodilation by changing the perfusion pressure and by the application of drugs.

In conclusion, using our new constant-pressure perfusion system for the rat mesentery with the intestine attached, we demonstrated that both mechanical and chemical stimulation can cause endothelium-dependent flow increases under tonic vasoconstriction. The pressure-induced flow increase may be partly mediated by NO but not by prostanoids or substance(s) opening charybdotoxin- and apamin-sensitive K^+ channels, whereas the agonist-induced flow increase was thought to be mainly mediated by substance(s) opening charybdotoxin- and apamin-sensitive K^+ channels, but not by NO or prostanoids. The opening of charybdotoxin- and apamin-sensitive K^+ channels stimulated by the agonists did not seem to be affected by mechanical stress on the endothelium.

Acknowledgements

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