—Review—

Endothelial Modulation of Agonist-induced Vasoconstriction in Mesenteric Microcirculation

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It is widely accepted that vascular endothelium regulates vasoconstriction *via* release of endothelium-derived relaxing factors (EDRF). The mesenteric circulation, which is the largest vascular bed, influences regulation of systemic blood pressure. However, the role of EDRF in the modulation of vascular tone in peripheral mesenteric circulation has not been extensively studied. Therefore, our recent studies investigated the role of the vascular endothelium in the regulation of methoxamine (α_1-adrenoceptor agonist)-induced vasoconstriction and their age-related changes in rat mesenteric vascular beds. In mesenteric vascular beds with intact endothelium isolated from 8 week-old rats, the initial maximum vasoconstriction induced by continuous perfusion of methoxamine was time-dependently decreased during 3 hour-perfusion. Neither nitric oxide synthase inhibitor nor cyclooxygenase inhibitor altered this time-dependent reduction of methoxamine-induced vasoconstriction. Endothelium removal, K^+-channel inhibitors and gap junction inhibitor significantly inhibited the time-dependent reduction of methoxamine-induced vasoconstriction. In the preparations with intact endothelium from 16 week-old rats, the time-dependent reduction of methoxamine-induced vasoconstriction disappeared. Furthermore, endothelium removal and treatment with cyclooxygenase inhibitor, thromboxane A_2 receptor antagonist or superoxide dismutase mimetic significantly reduced the methoxamine-induced vasoconstriction in the preparations from 16 week-old rats. These findings suggest that vascular endothelium acts to depress methoxamine-induced vasoconstriction by releasing endothelium-derived hyperpolarizing factor (EDHF), and dysfunction in this endothelial modulation develops with ageing.

Key words——agonist-induced vasoconstriction; endothelium-derived hyperpolarizing factor; ageing; mesenteric resistance artery

INTRODUCTION

Vascular endothelium is a layer of flat thin cells, which line at the luminal surface of blood vessels, and has a large surface area for exchange of materials between blood and tissues. Initially, the physiological role of endothelium has been considered to prevent aggregation of blood products as an antithrombotic surface and regulate exchange of certain molecules as a barrier between plasma and tissue. In recent decades, it has become clear that endothelium cells can be activated by various chemical stimuli including endogenous (acetylcholine, 5-hydroxutriptamine, bradykinin, and histamine) and exogenous (adrenoceptor agonists, nucleotides) vasoactive substances or physical stimuli (shear stress and pulsatile stretch) (Fig. 1). In response to these stimulations, the endothelium releases endothelium-derived vasoactive factors such as relaxing factors (EDRF) and contracting factors (EDCF).[1,2] EDCF and EDRF include endothelin, prostaglandin $F_{2\alpha}$ and thromboxane A_2 (TXA_2) and nitric oxide (NO), prostaglandin I_2 (PGI_2) and endothelium-derived hyperpolarizing factor(s) (EDHF), respectively (Fig. 1).

Vascular smooth muscles in physiological conditions are always exposed to vasoconstrictor stimuli from the outside adventitia *via* perivascular sympathetic adrenergic nerves, which release neurotransmitter noradrenaline to produce active tone by activating α_1-adrenoceptors on smooth muscle cells, and also exposed by various vasoactive substances, such as endogenous vasoconstrictor substances including α_1-adrenoceptor agonists (catecholamines) and serotonin (5-hydroxytryptamine, 5-HT), in plasma in physiological state.[3] As shown in Fig. 2(A), in mesenteric vascular beds with intact endothelium isolated from male Wistar rats, periarterial nerve stimulation (PNS) induced frequency-dependent increase

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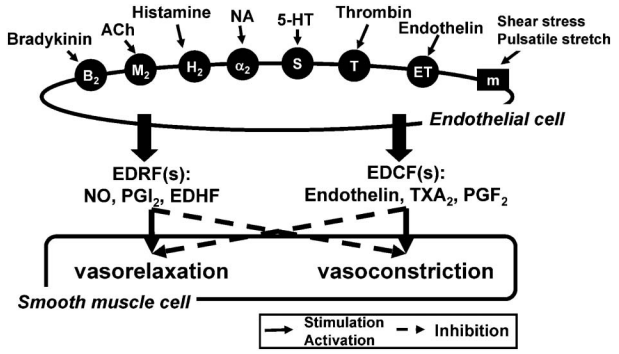

Fig. 1. Endothelium-derived Relaxing and Contracting Factors

Endothelium-derived relaxing factors (EDRFs) and contracting factors (EDCFs) are released by physical stimuli and various vasoactive substances through their receptors. ACh, acetylcholine; α_2, alpha-2 adrenergic receptor; B_2, bradykinin receptor; ET, endothelin receptor; H_2, histaminergic receptor; 5-HT, serotonin (5-hydroxytryptamine); S, serotoninergic receptor; M_2, muscarinic receptor; NA, noradrenaline (norepinephrine); NO, nitric oxide; PGI_2, prostaglandin I_2; EDHF, endothelium-derived hyperpolarizing factor; PGF_2, prostaglandin $F_{2\alpha}$; TXA_2, thromboxane A_2.

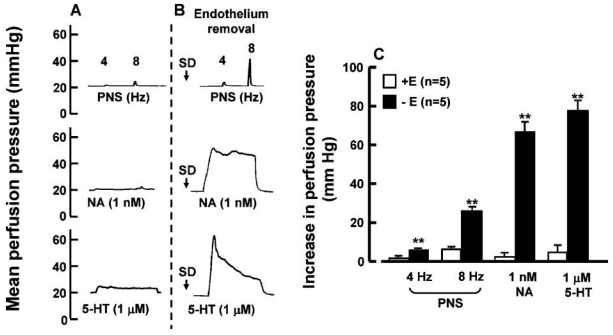

Fig. 2. The Role of Endothelium in Vasoconstriction

Typical records showing the vasoconstrictor responses to periarterial nerve stimulation (PNS, 4 and 8 Hz), perfusion of noradrenaline (NA, 1 nM) and 5-hydroxytryptamine (5-HT, 1 μM) in rat perfused mesenteric vascular beds with (A) and without (B) intact endothelium, respectively. A bar graph (C) showing the effects of endothelium removal on vasoconstrictor responses to periarterial nerve stimulation (PNS, 4 and 8 Hz), perfusion of noradrenaline (NA, 1 nM) and 5-hydroxytryptamine (5-HT, 1 μM) in rat perfused mesenteric vascular beds with resting tone. +E, with intact endothelium. −E, endothelium removal. SD, perfusion of sodium deoxycholate. Data indicate mean±S.E.M. $^{**}p<0.01$, compared with (+E).

in perfusion pressure due to vasoconstrictions. However, the responses were very small. Perfusion of 1 nM noradrenaline or 1 μM 5-HT also produced a small increase in perfusion pressure due to a slight vasoconstriction (Fig. 2(A)). After chemical removal of endothelium by sodium dexycholate (SD), vasoconstriction induced by PNS, noradrenaline or 5-HT was markedly augmented (Fig. 2(B) and (C)). These findings strongly suggest that the endothelium plays a major inhibitory role in vasoconstriction in mesenteric arteries to normalize vascular tone by releasing EDRF.

The Role of EDRF in α_1-Adrenoceptor Agonist-induced Vasoconstriction

As shown in Fig. 3(A), in the rat mesenteric vascular beds with an intact endothelium form 8 week-old male Wistar rats, continuous perfusion of methoxamine (α_1-adrenoceptor agonist), caused a long-lasting vasoconstriction. However, the vasoconstriction gradually decreased during 180-min perfusion in a time-dependent manner, causing 80% reduction of the initial constriction at 180 min (Fig. 3(C)). In contrast, perfusion of Krebs solution with high K^+-medium (high KCl) induced vasoconstriction, which were maintained over 180 min throughout the experiment and no time-dependent reduction was observed (Fig. 3(B)). After removal of the endothelium, methoxamine-induced vasoconstriction was sustained over 180 min and the reduction of vasoconstriction was significantly inhibited (Fig. 3(C)), suggesting that the endothelium plays an important inhibitory role in vasoconstriction induced by methoxamine. Methoxamine selectively stimulates α_1-adrenoceptors to induce direct constriction of vascular smooth muscle, which has been shown to have no direct action on the endothelial cells.[4] It is the most likely that methoxamine-induced vasoconstriction in isolated mesenteric resistance arteries results in increased endothelial mechanical stimuli, which stimulate the endothelium and elicit the release of EDRF. In contrast, high KCl causes depolarization of smooth muscle cells and induces vasoconstriction without stimulation of receptors, and the ability of K^+-channels is blunted in high KCl. Therefore, it is likely that EDRFs, *via* activating K^+-channels, are responsible

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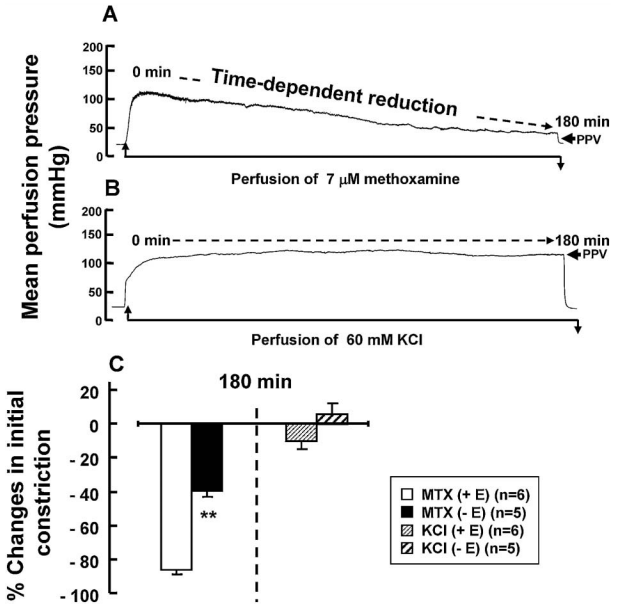

Fig. 3. Vasoconstritor Responses to Perfusion of Methoxamine and High KCl

Typical records showing changes in perfusion pressure following perfusion of 7 μM methoxamine (A) and 60 mM KCl (B) for 180 min in perfused mesenteric vascular beds with and without intact endothelium from 8-week old rats. A bar graph (C) showing percent changes in methoxamine- and KCl-induced maximum perfusion pressure increase at 180 min in preparations with and without intact endothelium from 8-week old rats. Time 0 indicates the maximum increase in perfusion pressure after starting continuous perfusion of methoxamine or high KCl. PPV, perfusion of 100 μM papaverine. MTX, methoxamine. (+E), with intact endothelium. (−E), endothelium removal. Data indicate mean±S.E.M. $^{**}p<0.01$, compared with MTX (+E).

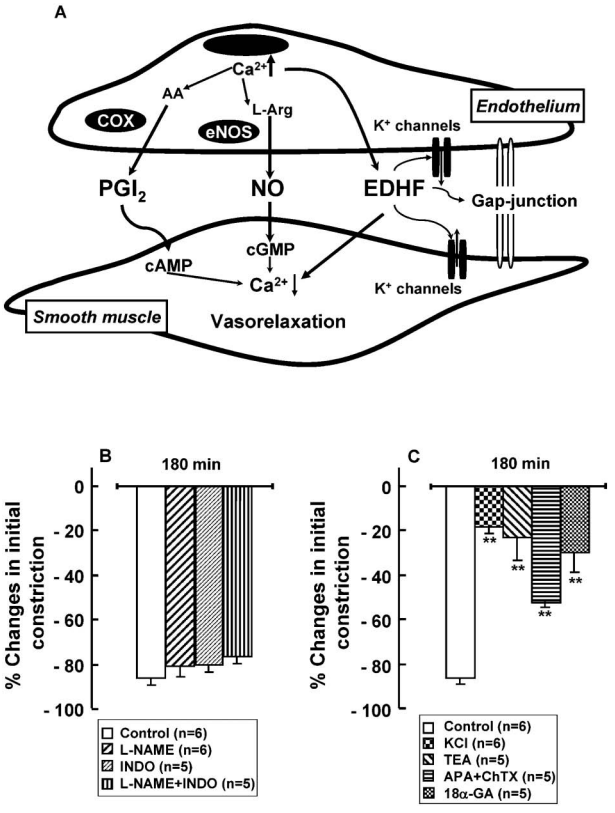

Fig. 4. The Role of EDRF in Methoxamine Induced Vasoconstriction

Schematic drawing (A) of possible mechanisms underlying vasorelaxation induced by nitric oxide (NO), prostaglandin I_2 (PGI_2), and endothelium-derived hyperpolarizing factors (EDHFs). Bar graphs (B and C) showing the effects of N^G-nitro-L-arginine-methyl ester (L-NAME; 100 μM), indomethacin (INDO; 1 μM), high KCl (30 mM), tetraethylammonium (TEA; 5 mM), 10 nM apamin plus 10 nM charybdotoxin (APA+ChTX) and 18α-Glycyrrhetinic acid (18α-GA; 10 μM) on percent changes in methoxamine-induced maximum perfusion pressure increase at 180 min in perfused mesenteric vascular beds with intact endothelium from 8-week old rats. AA, arachidonic acid; cAMP, cyclic-AMP; cGMP, cyclic-GMP. COX, cyclooxygenase; eNOS, endothelial nitric oxide synthase; L-Arg, L-arginine. Data indicate mean±S.E.M. $^{**}p<0.01$, compared with control.

for the time-dependent reduction of methoxamine-induced vasoconstriction.

Vascular endothelial cells generate three main EDRFs including prostacyclin (PGI_2), nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF).[1,2] PGI_2, a metabolite of arachidonic acid produced by cyclooxygenase in endothelial cells, activates inositol phosphate (IP) receptors on vascular smooth muscles and relaxes most of the arteries.[5] NO, a main EDRF, activates directly soluble guanylate cyclase in vascular smooth muscle cells and increases intracellular cyclic GMP production, resulting in vasorelaxation. EDHF is an endothelium-derived non-NO and non-PGI_2 factor, but the nature of EDHF is still unknown (Fig. 4(A)). As shown in Fig. 4(B), the time-dependent reduction of vasoconstriction induced by methoxamine was not altered by indomethacin, a prostanoid synthase cyclooxygenase inhibitor, or the NO synthase inhibitor, L-NAME. Several studies have reported that indomethacin inhibits endothelium-dependent vasorelaxation in the aorta of rats and mouse, suggesting that the endothelium regulates vasoconstriction by releasing EDRF including NO and PGI_2 in the aorta.[6–8] However, it is unlikely that prostanoid EDRF or NO released from the endothelium is involved in time-dependent reduction of methoxamine-induced vasoconstriction in rat mesenteric arteries. EDHF, which is described as an endothelium-derived non-NO and non-PGI_2 factors, is still unknown. EDHF hyperpolarizes vascular smooth muscle cell membranes by opening K^+ channels. Many studies suggested that K^+,[9,10] cytochrome P450 metabolites[11] or H_2O_2[12] has been proposed to be a most possible EDHF in several arteries. In general, EDHF−mediated responses involve an increase in the intracellular calcium concentration in the endothelial cells, the activa-

tion of calcium-activated potassium channels and the hyperpolarization in the smooth muscle cells. In the present study, as shown in Fig. 4(C), the reduction of methoxamine-induced vasoconstriction was significantly inhibited by K^+-channel blockers, including tetraethylammonium (TEA), apamin (APA) plus charybdotoxin (ChTX). The combination of APA (selective inhibitor for SK_{Ca} channels) plus ChTX (selective inhibitor for IK_{Ca} channels) has been reported to attenuate EDHF relaxations in arteries of several species (rat, dog and pig).[13–16]

The endothelium and smooth muscle cells have been shown to communicate *via* myo-endothelial gap-junctions, which are thought to have an important role in the EDHF response.[17–19] In many studies, glycyrrhetinic acid (GA) derivatives, such as 18α-GA, was used to disrupt myo-endothelial gap-junctions,[20,21] and this inhibitor has been shown to block endothelium-dependent hyperpolarization and relaxation in blood vessels and vascular beds.[22] The present study using gap-junction inhibitor showed that 18α-GA resulted in marked inhibited the reduction of methoxamine-induced vasoconstriction (Fig. 4(C)). Evidence has accumulated that NO and PGI_2 of EDRF play a prominent role in the tone control of large conduit arteries, whereas EDHF plays a major role in the distal mesenteric arteries in response to vasoactive substances or physical stimuli such as shear stress and pulsatile stretch.[23] Therefore, from these findings of our recent studies suggest that the endothelium acts to depress methoxamine-induced long-lasting vasoconstriction by releasing EDHF, which is associated with activation of multiple K^+-channels and involvement of gap-junctions in rat mesenteric resistance arteries.

Age-related Disappearance of the Endothelial Modulation of Agonist-induced Vasoconstriction

Age-related alteration in vasoconstrictor response to various vasoconstrictor responses including noradrenaline, 5-HT and endothelin-1 have been studied in humans and experimental animals, resulting in age-related reduction of endothelial regulation of vasoconstriction.[24] As shown in Fig. 5(A), in rat mesenteric arteries with intact endothelium of 16 week-old, continuous perfusion methoxamine also caused a long-lasting increase in perfusion pressure due to vasoconstriction, which was maintained over 180 min throughout the experiment and rather increased gradually. The time-dependent reduction of methoxamine-induced vasoconstriction, which observed in the preparations of 8 week-old, was disappeared (Fig. 5(A) and (B)). However, high KCl-induced vasoconstriction did not alter with ageing, suggesting that this is not due to changes in the smooth muscle contractility. Thus, it is likely that the disappearance of time-dependent reduction of methoxamine-induced vasoconstriction in preparations from 16 week-old rats is due to the marked reduction and or abolition of inhibitory effect of vascular endothelium in rat mesenteric resistance arteries with ageing.

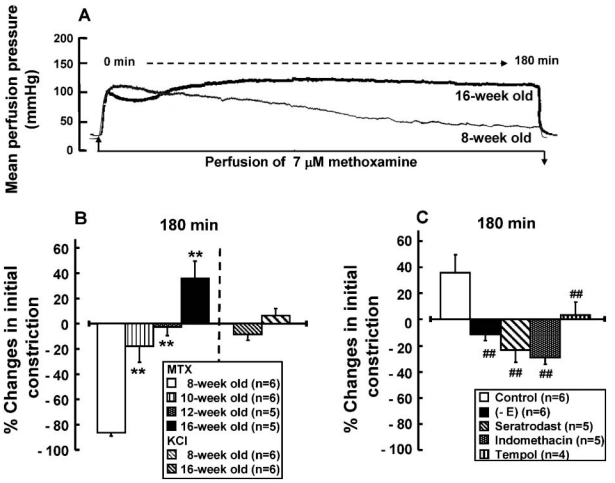

Fig. 5. Age-related Changes in Vasoconstriction Induced by Methoxamine

Typical records (A) showing changes in perfusion pressure following perfusion of 7 μM methoxamine at concentrations of for 180 min in perfused mesenteric vascular beds with an intact endothelium isolated from 8- and 16-week old rats. A bar graph (B) showing percent changes in methoxamine- and KCl-induced maximum perfusion pressure increase at 180 min in perfused mesenteric vascular beds with intact endothelium from 8-, 10-, 12- and 16-week old rats. A bar graph (C) showing the effects of endothelium removal, indomethacin (1 μM), seratrodast (1 μM), and tempol (100 μM) on percent changes in methoxamine-induced maximum perfusion pressure increase at 180 min in perfused mesenteric vascular beds with intact endothelium from 16-week old rats. (−E), endothelium removal. Data indicate mean±S.E.M. $^{**}p<0.01$, compared with 8-week old. $^{\#\#}p<0.01$, compared with control (16-week old).

On the other hand, in rat mesenteric arteries from 16 week-old rats, methoxamine-induced vasoconstriction was significantly attenuated by removal of endothelium, indomethacin (prostanoid synthase cyclooxygenase inhibitor), seratrodast (TXA_2 receptor antagonist) or tempol (SOD mimetic). It has been reported that ageing or pathological conditions augments the release of EDCFs and reactive oxygen species (ROS).[25–28] Taken together, therefore, the present findings suggest that released EDCF and ROS may in part contribute to age-related alteration of

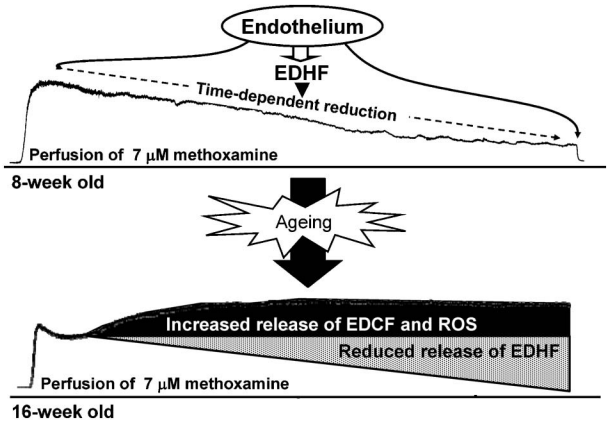

Fig. 6. Possible Mechanisms of Endothelial Modulation of Agonist-induced Vasoconstriction

Schematic drawing of possible mechanisms underlying the inhibitory effect of vascular endothelium on methoxamine-induced vasoconstriction and the age-related change of this endothelial modulation of agonist-induced vasoconstriction in rat mesenteric resistance arteries. EDCF, endothelium-derived contracting factors; EDHF, endothelium-derived hyperpolarizing factors; ROS, reactive oxygen species.

methoxamine-induced vasoconstriction in 16 week-old rats.

CONCLUSION

In mesenteric microcirculation, vascular endothelium acts to depress agonist-induced vasoconstriction by releasing EDRF, mainly EDHF, which is associated with activation of multiple K^+-channels and gap junction. Furthermore, this inhibitory effect of vascular endothelium on agonist-induced vasoconstriction disappears with ageing and that this is likely due to reduced EDHF and increased EDCF and in part ROS (Fig. 6).

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