Endothelium-Dependent Changes in the Response to Vasoconstrictor Substances of Isolated Dog Mesenteric Veins

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Abstract—In dog mesenteric vein strips, contractions induced by histamine relative to those induced by 5 mM Ba** were potentiated by removal of endothelium. The induced contractions were potentiated by AA861, a lipoxygenase inhibitor, and methylene blue, a guanylate cyclase inhibitor, to an appreciably greater extent in the strips with endothelium than in those with damaged endothelium. Indomethacin did not potentiate the contraction induced by histamine. Cimetidine potentiated the contraction in control strips and those without endothelium to a similar extent whereas chlorpheniramine suppressed the contraction. Contractile responses to acetylcholine, norepinephrine, serotonin and prostaglandin (PG) F12a were not potentiated by removal of endothelium. It may be concluded that histamine activates histaminergic receptors, possibly H1 but not H2, in endothelial cells and results in a release of vasodilator substance produced by lipoxygenase, which accumulates cellular cyclic GMP and relaxes mesenteric veins. The H1 and H2 receptors in smooth muscle cells appear to be responsible for contractions and relaxations, respectively. Acetylcholine, norepinephrine, serotonin and PGF12a do not seem to release vasodilator substances from endothelium in an amount sufficient to cause significant relaxations of venous smooth muscle.

Vascular endothelium plays an important role in vasodilatation induced by chemical substances, including acetylcholine, bradykinin, angiotensin II, substance P, histamine, ATP, thrombin and Ca++ ionophore (1). Vasodilator substances such as cyclooxygenase products, mainly prostaglandin (PG) I2 (2-4), lipoxygenase products (5, 6), and others (5), are liberated by chemical stimulation of endothelial cells. Removal of endothelium, where the vasodilators are biosynthesized, is expected to abolish vasodilatation, to reverse vasodilatation to vasoconstriction or to potentiate vasoconstriction. Therefore, attention is currently directed to an impairment of endothelial cell function as a mechanism of localized spasm of conduit coronary and cerebral arteries.

Human coronary arteries respond to norepinephrine, histamine and acetylcholine with contractions, whereas monkey and dog coronary arteries respond to histamine and acetylcholine with relaxations (7). Norepinephrine causes slight contractions in monkey coronary arteries and relaxations in dog arteries (8). Among dog blood vessels, mesenteric vein strips are those responding to these agents with contractions, like human coronary arteries. Therefore, in the present study, contractile responses to norepinephrine, acetylcholine, histamine, PGF12a and serotonin were compared in control mesenteric vein strips and those from which the endothelium was removed.

Materials and Methods

Mongrel dogs of either sex, weighing 8 to 15 kg, were anaesthetized with intravenous injections of sodium thiopental (30 mg/kg) and killed by bleeding from the carotid arteries. The mesentery was rapidly removed. Superior mesenteric veins (1.5 to 2.0 mm
outside diameter) along with distal mesenteric arteries were isolated, and they were helically cut into strips, approximately 20 mm long. The vein strip was vertically fixed between hooks in a muscle bath containing the modified Ringer-Locke solution, which was aerated with a mixture of 95% O₂ and 5% CO₂ and maintained at 37±0.3°C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon-Kohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 0.7 g, which was optimal in producing the maximal contraction. Constituents of the solution were as follows (mM): NaCl, 120; KCl, 5.4; NaHCO₃, 25.0; CaCl₂, 2.2; MgCl₂, 1.0 and dextrose, 5.6. The pH of the solution was 7.35 to 7.41. The strips were allowed to equilibrate for 60 to 90 min in the bathing media, during which time the solution was replaced every 10 to 15 min.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Nihon-Kohden Kogyo Co.). The contractile response to 30 mM K⁺ was first obtained, and the preparations were washed repeatedly. The response to 5 mM Ba²⁺ was then obtained; the contraction averaged 446±26 mg (N=51). The mean value of K⁺-induced contractions was 51.7±2.8% (N=51) of the contractions induced by Ba²⁺. Cumulative concentration-response curves for norepinephrine, acetylcholine, histamine, PGF₂α and serotonin were obtained under resting conditions by adding the compounds directly to the bathing media. Contractions by test drugs relative to those induced by 5 mM Ba²⁺ are presented. In the experiments, in which the responses were obtained in the presence of pharmacological antagonists, the strips were exposed for 20 to 30 min to the antagonists prior to the addition of test drugs.

The intimal surface of vein strips was gently rubbed with a cotton pellet to remove endothelial cells (6); unrubbed strips obtained from the same dogs were used for comparison. Removal of endothelium was determined by AgNO₃ staining (9). The intimal surface in control and rubbed strips is histologically compared in Fig. 1.

The results shown in the text, table and

![Fig. 1. Intimal surface stained by AgNO₃ of mesenteric vein strips obtained from the same dog. Left picture, control strip; right, strip in which the intimal surface was gently rubbed with a cotton pellet.](image-url)
figures are expressed as mean values±S.E.M. Statistical analyses were made using Student's paired and unpaired t-test. Drugs used were dl-norepinephrine hydrochloride, acetylcholine chloride, histamine dihydrochloride, serotonin creatinine sulfate, PGF$_{2\alpha}$, diphloretin phosphate (Ono Pharmaceutical Co., Osaka), indomethacin, d-chlorpheniramine maleate, cinanserin, cimetidine, AA861 (2,3,5-trimethyl-6-[12-hydroxy-5,10-dodecadiynyl]-1,4-benz-oquinone, Takeda Chemical Industries, Ltd., Osaka), ETYA (5,8,11,14-eicosatetraynoic acid), methylene blue trihydrate (Nakarai Chemicals, Ltd., Kyoto, Japan), atropine sulfate and papaverine hydrochloride.

**Results**

The addition of norepinephrine (2×10$^{-8}$ to 10$^{-5}$ M) produced a concentration-

| Agent            | ED50 (M) | Maximum contraction (%)$^a$ |
|------------------|----------|----------------------------|
| Norepinephrine   | 16       | 282±14.0 278±22.8          |
| Acetylcholine    | 14       | 161.2±10.1 188.7±17.5      |
| Histamine        | 18       | 139.8±13.4 188.1±20.4      |
| PGF$_{2\alpha}$  | 10       | 184.9±13.5 221.5±26.0      |
| Serotonin        | 10       | 65.4±5.2 67.5±5.8          |

* Maximum contractions relative to those induced by 5 mM Ba$^{2+}$. N, number of preparations used.

$^a$ Significantly different from the values in strips with endothelium, P<0.05.

**Fig. 2.** Concentration-contraction response curves for acetylcholine (left figure) and histamine (right) in control mesenteric vein strips and those from which the endothelium was removed. Contractions induced by 5 mM Ba$^{2+}$ were taken as 100%; mean absolute values in control and rubbed strips were 483±51 and 386±51 mg (N=14), respectively, in the experiments with acetylcholine, and they were 402±40 and 413±55 mg (N=18), respectively, in the experiments with histamine. $^a$ Significantly different from the values in strips with endothelium. P<0.01; $^b$ P<0.05. Vertical bars represent the S.E.M.
dependent contraction of mesenteric vein strips under resting conditions. Maximum contractions induced by 10^{-6} M norepinephrine relative to Ba^{++} (5 mM)-induced contractions were the greatest among those induced by the vasoconstrictors used (Table 1). Removal of endothelium did not alter the contraction induced by norepinephrine and its apparent median effective concentration (ED50) (Table 1). Treatment with prazosin (10^{-9} M) or yohimbine (10^{-8} M) significantly attenuated the amine-induced contraction, suggesting the involvement of \( \alpha_1 \) and \( \alpha_2 \) adrenoceptor subtypes (10).

Contractile responses to acetylcholine (10^{-7} to 10^{-8} M) were not significantly altered by removal of endothelium (Fig. 2, left). Treatment with 10^{-7} M atropine suppressed the acetylcholine-induced contraction (N=5). Indomethacin (10^{-6} M) did not significantly alter the contraction induced by acetylcholine (N=5). Treatment with methylene blue (10^{-5} M) did not increase the contraction; mean values of the contraction at 10^{-4} M acetylcholine before and after the inhibitor were 304±44 mg and 330±50 mg (108±9.3%, N=13), respectively.

Removal of endothelium potentiated the contraction induced by histamine in concentrations ranging from 5\times10^{-7} to 2\times10^{-4} M (Fig. 2, right). Tachyphylaxis in the contractions developed; however, the response was reproducible after 3rd trials of increased amine concentrations (up to 10^{-5} M histamine). Therefore, the third concentration-response curve was taken as a control. Chlorpheniramine (10^{-6} M) abolished the histamine-induced contraction (N=4). Treatment with 10^{-5} M cimetidine significantly potentiated the contractile response in control strips and those from which the endothelium was removed (Fig. 3). On the other hand, AA861 (10^{-6} M), a 5-lipoxygenase inhibitor (11), or methylene blue, a guanylate cyclase

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Fig. 3. Potentiation by cimetidine of the contractile response to histamine in mesenteric vein strips with (left figure) and without endothelium (right). Contractions induced by 10^{-5} M histamine in control media were taken as 100%; mean absolute values in the control and rubbed strips were 305±59 mg (N=10) and 251±46 mg (N=10), respectively. a Significantly different from the control, \( P<0.001 \); b \( P<0.01 \); c \( P<0.02 \). Vertical bars represent the S.E.M. Numbers in parentheses indicate the number of preparations used.
Fig. 4. Potentiation by AA861 of the contractile response to histamine in mesenteric vein strips with (left figure) and without endothelium (right), treated with 10⁻⁵ M cimetidine. Contractions induced by 10⁻⁵ M histamine in control media containing cimetidine were taken as 100%; mean absolute values in the control and rubbed strips were 232±41 mg (N=8) and 226±59 mg (N=8), respectively. *Significantly different from the control, P<0.01; †P<0.05. Vertical bars represent the S.E.M.

Inhibitor (12), potentiated the response to histamine in control strips treated with cimetidine to an appreciably greater extent than in strips with damaged endothelium (Figs. 4 and 5). Typical recordings in the strips treated with methylene blue are shown in Fig. 6. Potentiation by these inhibitors persisted even after repeated washing of the preparations. Ba⁺⁺ (5 mM)-induced contractions were slightly inhibited by 10⁻⁵ M AA861 (19.0±5.1% decrease, N=9, P<0.01) and potentiated by 10⁻⁵ M methylene blue (14.6±6.0% increase, N=9, P<0.05). ETYA (10⁻⁵ M) reduced the contractile response to histamine (52.7±5.4% inhibition at 10⁻⁶ M histamine in 4 control strips) and that to PGF₂α (2×10⁻⁶ M), suggesting a non-selective inhibition in vascular contractility. Treatment with 10⁻⁶ M indomethacin did not alter or slightly attenuated the histamine-induced contraction in control strips treated with 10⁻⁶ M cimetidine; the mean contraction at 10⁻⁵ M histamine in the presence of the inhibitor was 85.7±6.2% (N=6) of the control, being not significantly different.

Concentration-contractile response curves for PGF₂α and serotonin were not significantly influenced by removal of endothelium; the ED₅₀ values and the maximum contractions in the presence and absence of endothelium are summarized in Table 1. Contractions induced by PGF₂α were suppressed by treatment with 10⁻⁵ M diphloretin phosphate (N=4), a PG antagonist (13), and serotonin-induced contractions were markedly attenuated by 10⁻⁵ M cinanserin (N=4).

Discussion

Dog mesenteric vein strips responded to norepinephrine, acetylcholine, histamine, PGF₂α, and serotonin with concentration-dependent contractions in a similar way to human coronary arteries (7, 14, 15). The contractile response to histamine was potentiated by removal of endothelium, whereas the response to other vasoconstrictors was unaffected. Contractions induced by histamine were potentiated by
Fig. 5. Potentiation by methylene blue of the contractile response to histamine in mesenteric vein strips with (left figure) and without endothelium (right), treated with $10^{-5}$ M cimetidine. Contractions induced by $10^{-5}$ M histamine in control media containing cimetidine were taken as 100%; mean absolute values in the control and rubbed strips were 226±38 mg (N=10) and 231 ±61 mg (N=10), respectively. a Significantly different from the control, $P<0.001$; $b P<0.01$; $c P<0.02$. Vertical bars represent the S.E.M.

AA861 and methylene blue in vein strips with endothelium to a markedly greater extent than in the strips with damaged endothelium. AA861 reportedly inhibits 5-lipoxygenase of peritoneal guinea pig polymorphonuclear leucocytes with an ID50 of 0.8 $\mu$M (11). Methylene blue, an inhibitor of guanylate cyclase (12), decreases the production of cellular cyclic GMP (16). These findings support the hypothesis that histamine liberates vasodilator substance produced by the catalysis of lipoxygenase from the endothelium, which acts on vascular smooth muscle to accumulate cyclic GMP, resulting in vasodilatation. Such a mechanism has been postulated in relaxations mediated by acetylcholine (16, 17). In contrast, indomethacin in an effective concentration ($10^{-8}$ M) failed to potentiate the histamine-induced contraction, suggesting that cyclooxygenase products are not involved. Cimetidine potentiated the histamine-induced contraction in control and rubbed strips to a similar extent, indicating the involvement of a mechanism other than that mediated by H2 receptors in the endothelium-dependent potentiation in contractile responses. Histamine-induced contractions were suppressed by an H1 receptor antagonist, chlorpheniramine. Endothelium is postulated to release PGI2-like substance from dog mesenteric arteries and human umbilical veins (18, 19) or non-PG vasodilator substance from rat aortae and guinea pig pulmonary arteries (20, 21) by activation of H1 receptors. Although direct evidence was not obtained, H1 receptors may participate in the release of vasodilator substance from the endothelium of dog mesenteric veins used in the present study.

Contractions induced by acetylcholine were not potentiated by removal of endothelium in dog mesenteric (present study), femoral, saphenous and splenic veins (22, 23) but were potentiated in dog pulmonary veins (22). Removal of endothelium is also ineffective in acetylcholine-induced con-
Fig. 6. Typical recordings of the response to histamine in the control and rubbed mesenteric vein strips before and after treatment with methylene blue. The strips were obtained from the same dog and treated with $10^{-5}$ M cimetidine. Concentrations of histamine from 1 to 5: $2 \times 10^{-8}$, $10^{-7}$, $5 \times 10^{-7}$, $2 \times 10^{-6}$ and $10^{-5}$ M, respectively.

The strips were obtained from the same dog and treated with $10^{-5}$ M cimetidine. Concentrations of histamine from 1 to 5: $2 \times 10^{-8}$, $10^{-7}$, $5 \times 10^{-7}$, $2 \times 10^{-6}$ and $10^{-5}$ M, respectively.

Potentiations of the contractile response to norepinephrine by removal of endothelium are observed in isolated dog and pig coronary arteries (24). These authors suggest the presence of $\alpha_2$ adrenoceptors in the arteries, which mediate an endothelium-dependent relaxation. However, in the present study, norepinephrine-induced contractions, mediated via $\alpha_1$ as well as $\alpha_2$ receptors (10), were not significantly increased by removal of endothelium. The amine-induced contraction in dog saphenous veins is also unaffected by endothelial denudation (23).

Serotonin reportedly produces endothelium-dependent relaxations in pig and dog coronary arteries (24, 25). However, in dog mesenteric veins, serotonin-induced contractions were not potentiated by removal of endothelium. In the vein strips used here, vasodilator substance in an amount sufficient to produce a significant relaxation does not appear to be released from the endothelium by norepinephrine and serotonin. It is intriguing to clarify whether this is due to the absence of $\alpha_2$ and serotonergic receptors in the endothelium or due to inability of these receptors to liberate vasodilator substance.

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