

The endothelium: a modulator of cardiovascular health and disease

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The endothelial cells lining the luminal surface of blood vessels are involved in numerous regulatory functions, such as control of vascular smooth muscle cell proliferation, adhesion of leukocytes and platelets, permeability, and inflammatory responses.

The endothelium also has thrombolytic and fibrinolytic properties. Its metabolic activity contributes to the regulation of the oxidation of plasma lipids, angiotensin II formation, and the degradation of circulating catecholamines and kinins. In addition, the endothelium plays an important role in the regulation of vascular smooth muscle tone by releasing both relaxing and contracting factors. Endothelium-dependent relaxations are mediated primarily by nitric oxide, but also by endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin. The contracting factors are endothelin-1, metabolites from the cyclooxygenase pathway, and superoxide anions.

Under physiological conditions, a precise and balanced release of relaxing and contracting factors ensures adequate organ perfusion.

However, this balance is altered in disease states such as atherosclerosis, diabetes, chronic heart failure, coronary artery disease, or hypertension, thereby contributing to the further development of vascular diseases.

Keywords: nitric oxide; endothelium-dependent hyperpolarization; endothelin; thromboxane; hypertension; atherosclerosis; endothelial dysfunction

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The endothelial cells that line the luminal surface of all blood vessels constitute an important organ. The endothelium is involved in numerous regulatory functions, such as the control of vascular smooth muscle proliferation, leukocyte and platelet adhesion, vascular permeability, and inflammation. It also has thrombolytic and fibrinolytic properties. In addition, its metabolic activity regulates oxidation of plasma lipids, angiotensin II formation, and the degradation of circulating catecholamines and kinins. This paper updates previous reviews and only cites a limited number of references. Readers are invited to look at the Bibliography of 100 Key References at the end of this issue for a more comprehensive selection of papers.

SELECTED ABBREVIATIONS AND ACRONYMS

| | |
|---------------|--|
| ADMA | asymmetrical dimethyl-L-arginine |
| CNP | C-type natriuretic peptide |
| EDCF | endothelium-derived contracting factor |
| EDHF | endothelium-derived hyperpolarizing factor |
| EDRF | endothelium-derived relaxing factor |
| FAD | flavin adenine dinucleotide |
| ICAM-1 | intercellular cell adhesion molecule-1 |
| L-NAME | L-nitro-arginine methyl ester |
| L-NMMA | <i>N</i> ^G -monomethyl-L-arginine |
| MCP-1 | monocyte chemoattractant protein-1 |
| NANC | nonadrenergic noncholinergic (nerves) |
| NF-κB | nuclear factor kappa B |
| NO | nitric oxide |
| PDGF | platelet-derived growth factor |
| TNF-α | tumor necrosis factor α |
| VCAM-1 | vascular cell adhesion molecule-1 |

In 1980, Robert Furchgott demonstrated that endothelial cells generate vasoactive substances.¹ This seminal observation was crucial to the understanding of the regulation of vascular smooth muscle tone. Furchgott's simple pharmacological experiment initiated countless studies on a wide variety of blood vessels, shedding new light on the physiological role of nitric oxide.

Although nitric oxide appears to be the major vasodilator released by endothelial cells in the vast majority of blood vessels, other substances, some of them still unknown, may play a role as well. Soon after Furchgott's discovery, it became clear that endothelial cells not only release relaxing factors, but also produce contracting substances.² This concept led to the discovery of the endothelin peptides in the late 80s.

The release of endothelium-derived vasoactive substances is not only triggered by acetylcholine, but also controlled by a host of neuromediators and by shear forces exerted by the blood flowing through the blood vessel.²⁻⁴ Under physiological conditions, a precise and balanced release of relaxing and contracting factors ensures adequate organ perfusion. However, this balance is altered in disease states such as atherosclerosis, diabetes, chronic heart failure, coronary artery disease, or hypertension. Although the role of endothelial vasoactive factors as primary initiators of vascular diseases is still under debate, their dysfunctional release or their inactivation can have serious consequences for the vascular wall.

ENDOTHELIUM-DERIVED RELAXING FACTORS

Acetylcholine and other mediators release endothelium-derived relaxing factor(s) (EDRF) in many arteries and veins from different species, including human blood vessels (Figure 1).¹ Endothelium-dependent relaxation appeared early in evolution, even in species with a primitive cardiovascular system, which implies that these endothelial mediators fulfill a primordial role. Identification of the factors mediating endothelium-dependent relaxation was made difficult because of their short half-lives under in vitro experimental conditions. Indeed, bioassay experiments demonstrating that EDRF could diffuse from the endothelium to the smooth muscle also showed that the relaxing activity of the perfusate from a blood vessel was quickly lost when the transit time between the donor segment and the detector (an artery without endothelium) exceeded a few seconds. These bioassay experiments permitted

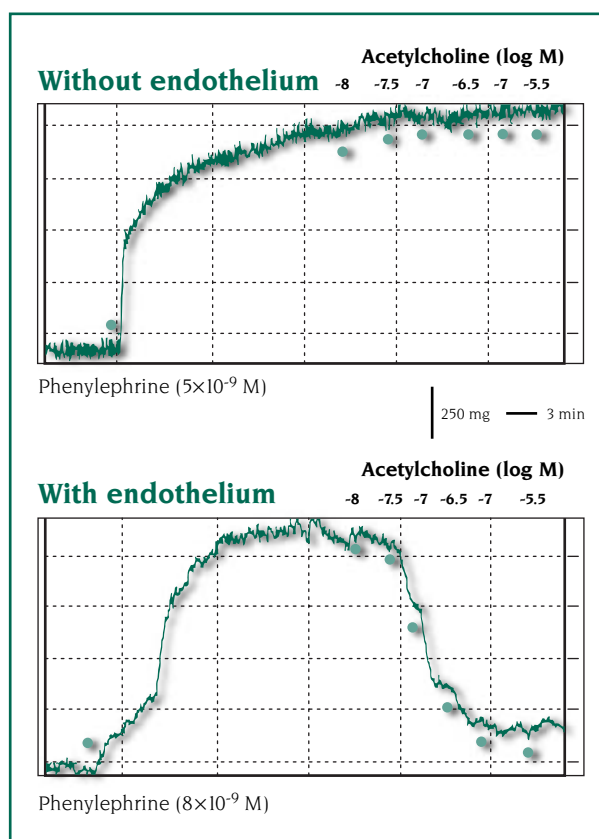


Figure 1. Typical recording showing the role of the endothelium in the regulation of vascular smooth muscle tone. The relaxation to acetylcholine (shown in the bottom trace) is lost after mechanically removing endothelial cells from the aorta of a mouse (top trace). The recording shows the points at which each agonist was added (in molar concentration). The preparations were first contracted with phenylephrine before exposure to increasing concentrations of acetylcholine. C.M. Boulanger, unpublished data.

the identification of the crucial role of superoxide anions in the inactivation of EDRF.

Nitric oxide

In 1986, Furchgott and Ignarro independently proposed nitric oxide, or a related nitroso compound, as the primary mediator of endothelium-dependent relaxation. This was based on pharmacological similarities between EDRF and nitric oxide, the active component of the nitrovasodilators, which have been used for therapy. Moncada and colleagues then identified nitric oxide (NO) as EDRF, which is synthesized following the conversion of endothelial L-arginine into L-citrulline.^{2,5,6}

Endothelium-dependent relaxation can be inhibited by analogs of L-arginine such as N^G-monomethyl-L-arginine (L-NMMA) or L-nitro-arginine methyl ester (L-NAME), which compete with the natural precursor

L-arginine at the catalytic site of the enzyme.⁶ When infused intravenously, L-NMMA induces a long-lasting increase in blood pressure. This suggests that the continuous basal release of NO by the endothelium contributes to the regulation of peripheral resistance. Indeed, mice lacking the gene for the endothelial isoform of NO synthase (type III) have a slightly higher arterial blood pressure than wild-type animals.

Mechanisms of NO release from the endothelium

The endothelial enzyme that produces NO (type III NO synthase) is a reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxygenase whose mechanism of action involves the oxidation of one of the guanidine nitrogen atoms of L-arginine.^{5,6} This NO synthase is located in the caveolae of the plasma membrane of endothelial cells (*Figure 2*).

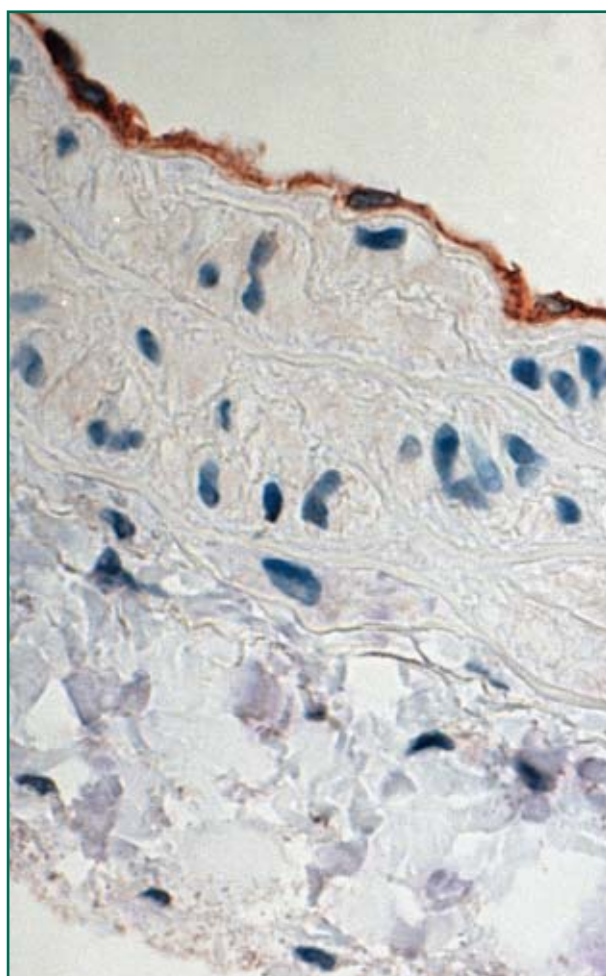


Figure 2. Immunohistochemistry showing the expression of endothelial type III NO synthase restricted to the endothelium in a rat carotid artery. Courtesy of Dr R.S. Geske, Baylor College of Medicine, Houston, Tex, USA.

The production of NO by the endothelium requires the enzyme to be membrane-bound as well as the presence of cofactors (such as tetrahydrobiopterin, flavin adenine dinucleotide (FAD), and flavin mononucleotide), and substrate (L-arginine).^{7,8} The enzyme binds calmodulin in a calcium-dependent manner and can therefore be activated by stimuli that increase the concentration of intracellular free calcium (such as acetylcholine or bradykinin). However, the release of endothelial NO can also occur in the absence of increase in intracellular free calcium, as observed in endothelial cells exposed to shear stress.⁹

Under normal conditions, the availability of the semi-essential amino acid L-arginine in the plasma (about 100 to 200 $\mu\text{mol/L}$) does not appear to be a limiting factor for the production of NO by type III NO synthase (K_m^{arg} : 5 to 10 $\mu\text{mol/L}$). Endothelial cells not only take up circulating L-arginine by means of the y^+ transporter but can also recycle L-citrulline into L-arginine. Endogenous inhibitors of NO synthase such as asymmetrical dimethyl-L-arginine (ADMA) or L-NMMA may interfere with L-arginine uptake and activation of the NO synthase(s), but the degree of NO synthase activation will depend upon the L-arginine: (ADMA + L-NMMA) ratio. The circulating levels of these endogenous inhibitors appear augmented in renal failure and in animal models of atherosclerosis.

Although type III NO synthase is also called “constitutive” NO synthase, the level of its messenger RNA (mRNA) can be regulated.^{6,7,10} Indeed, type III NO synthase mRNA levels appear to be augmented by shear stress and by exposure to vascular endothelial cell growth factor. Estrogens may also regulate the expression of this endothelial isoform, although this has not been observed consistently. In addition, type III NO synthase mRNA is decreased by oxidized LDL, while tumor necrosis factor α (TNF- α) shortens its half-life. This could contribute to pathophysiological changes in NO production by the blood vessel wall. Furthermore, the subsequent production of endothelial NO may be affected by changes in membrane localization of the enzyme (which is regulated by posttranslational palmytoylation and myristoylation) or its interaction with caveolin-1, as well as by alterations in the availability of L-arginine or the unavailability of cofactors.^{7,10,11} Finally, the amount of active NO released by endothelial cells depends on the level of superoxide anions that are able to degrade the mediator. Therefore, alteration of these different steps involved in the activation of endothelial NO release may contribute to the development of vascular disease.

Other NO synthase isoforms in the vessel wall

Another calcium-dependent NO synthase (type I) is expressed in nitrergic neurons innervating the adventitia of some cerebral arteries. Its role in the regulation of these blood vessels is not fully elucidated. NO released following activation of type I NO synthase is the mediator of the nonadrenergic noncholinergic innervation (NANC nerves).

Exposure to cytokines or lipopolysaccharides induces the expression of a calcium-independent NO synthase (type II) in the vessel wall.^{7,10} Once induced, this isoform spontaneously releases large and sustained amounts of the mediator, which may in part explain the hyporeactivity to contractile agents observed in septic shock. However, this type II isoform is "constitutively" expressed in the renal medulla, where it may participate in the control of arterial blood pressure.

Effects of endothelial NO

Relaxation of vascular smooth muscle

Endothelial NO causes the relaxation of vascular smooth muscle and can be regarded as a physiological antagonist of endogenous vasoconstrictors such as catecholamines, angiotensin II, or endothelin-1. NO stimulates soluble guanylate cyclase and augments the levels of cyclic guanosine monophosphate (GMP) in vascular smooth muscle cells (*Figure 3*).¹² This increase in cyclic GMP could contribute to the relaxation of the smooth muscle by decreasing Ca²⁺ influx, increasing calcium uptake by Ca²⁺ ATPases, or by a direct interaction at the level of contractile proteins. In some blood vessels, cyclic GMP specifically inhibits phosphodiesterase activity and may prevent the degradation of cyclic AMP, thus augmenting cyclic AMP-mediated relaxations. In addition, NO may cause relaxation by interacting with

K⁺ channels either directly or through an increase in cyclic GMP, as observed in the rabbit and rat aorta.^{13,14}

In addition to directly relaxing vascular smooth muscle, NO also regulates the production of the potent vasoconstrictor endothelin-1 in endothelial cells (*Figure 4*).¹⁵ This effect is mediated by an increase in cyclic GMP and is also observed with atrial natriuretic factor.

NO can also regulate the tone of vascular smooth muscle following a complex interaction with hemoglobin from red blood cells, glutathione, and small erythrocytic thiols. Release of oxygen to tissue is accompanied by the transfer of NO, bound to a cysteine group in hemoglobin, to small erythrocytic thiols where it can be released to cause relaxation of blood vessels. These data suggest that NO may be more than a purely local regulator of vascular smooth muscle tone, as previously thought.

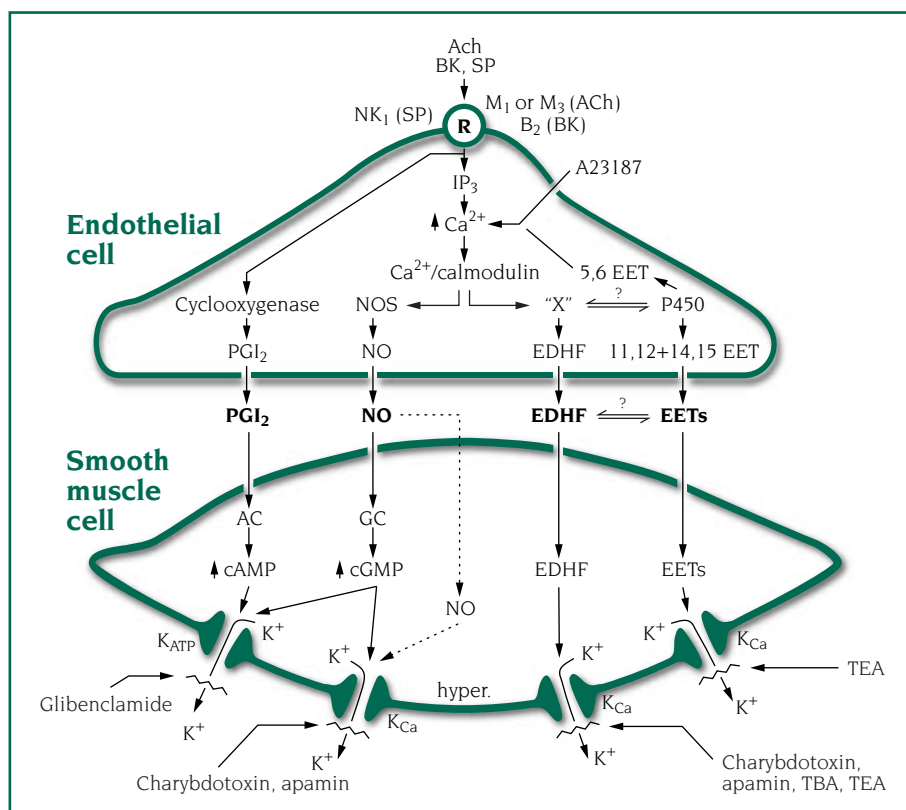


Figure 3. Schematic representation of the release of endothelium-derived relaxing factors from endothelial cells and their effect on vascular smooth muscle cells. AC, adenylate cyclase; Ach, acetylcholine; A23187, calcium ionophore A23187; BK, bradykinin; B₂, bradykinin B₂ receptor; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; EDHF, endothelium-derived hyperpolarizing factor; EET, epoxyeicosatrienoic acid; GC, guanylate cyclase; IP₃, inositol triphosphate; K_{ATP}, K_{Ca} potassium channels; M₁, M₃, muscarinic M₁ or M₃ receptor subtype; NK₁, neurokinin receptor; NO, nitric oxide; NOS, nitric oxide synthase; PGI₂, prostacyclin; P450, cytochrome P450; SP, substance P; TBA, tetrabutylammonium; TEA, tetraethylammonium. The broken line indicates the action of an inhibitor or an antagonist. Reproduced from ref 12: Vanhoutte PM. Endothelial dysfunction and inhibition of converting enzyme. Eur Heart J. 1998;19(suppl J): J7-J15. Copyright © 1998, The European Society of Cardiology. With permission.

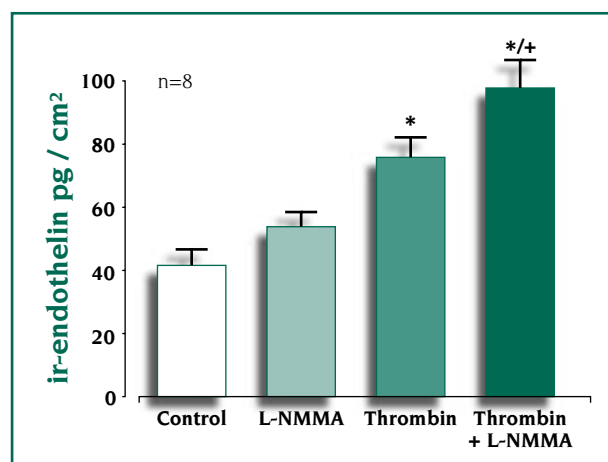


Figure 4. Inhibition by nitric oxide (NO) of the release of endothelin-1 from the porcine aorta. The NO synthase inhibitor L-NMMA (2×10^{-4} M) augments the release of endothelin following stimulation with thrombin (4 U/mL), demonstrating that NO exerts a negative regulatory feedback on the release of the peptide. The amount of peptide produced is expressed as picograms immunoreactive (ir) endothelin released per square cm intimal surface after 4 h incubation ($n=8$). * indicates a significant difference as compared with control; + indicates a significant difference of preparations stimulated with thrombin in the presence and absence of L-NMMA ($P < 0.05$). Reproduced from ref 15: Boulanger C, Luscher TF. Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide. *J Clin Invest.* 1990;85:587-590. Copyright © 1990, The American Society for Clinical Investigation, Inc. With permission.

Antiproliferative effects?

Endothelial NO may contribute to inhibition of the proliferation of vascular smooth muscle cells, as suggested by coculture experiments. However, the antiproliferative effect of NO and NO donors observed in cultured vascular smooth muscle cells is most convincing at high concentrations, which appear to be more compatible with the release of NO from an inducible NO synthase. Moreover, the antiproliferative effect of NO may depend upon the growth factors present in the medium, since culture experiments suggest that NO in the presence of fibroblast growth factor may actually stimulate vascular smooth muscle proliferation.

NO may have indirect antiproliferative effects, as it also downregulates the production of growth factors by endothelial cells such as endothelin-1 and the platelet-derived growth factor (PDGF) B-chain. This effect appears to be mediated by an increase in cyclic GMP.

Protective role and antiatherogenic effect

Soon after the demonstration that NO could mediate endothelium-dependent relaxation, it was observed that NO, in synergy with prostacyclin, prevents platelet

adhesion and aggregation at the endothelial surface. By inhibiting the degranulation of platelets, NO prevents the release of vasoconstrictors and growth factors such as thromboxane A_2 , serotonin, and PDGF.

By impairing the activation of nuclear factor kappa B (NF- κ B), NO negatively regulates the expression of adhesion proteins (such as vascular cell adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1], and monocyte chemoattractant protein-1 [MCP-1], the latter being a chemokine mediator of inflammation involved in the recruitment of circulating monocytes. NO, therefore, has a protective role against the initial events involved in cellular adhesion, which precedes the formation of macrophages/foam cells and the development of subsequent atherosclerotic lesions. In addition, NO prevents the oxidation of low-density lipoproteins, another crucial event in the development of atherosclerosis.

Endothelium-derived hyperpolarizing factor(s)

Endothelium-dependent hyperpolarization has been observed in a large variety of blood vessels, including human coronary arteries. Although endothelium-derived NO itself can cause hyperpolarization of vascular smooth muscle cells, endothelium-dependent hyperpolarization is mediated mainly by a distinct diffusible substance termed endothelium-derived hyperpolarizing factor (EDHF) (Figure 3). The identity of EDHF is unknown, although several candidates have been proposed, such as ammonium anions, cytochrome P450 metabolites, hydrogen peroxide, cannabinoids, and potassium ions (see also the article by M. Félétou, in this issue).

The intracellular pathway leading to EDHF synthesis is also unclear, but is likely to involve an increase in intracellular calcium, since the calcium ionophore A23187 causes endothelium-dependent hyperpolarization and the latter is absent in calcium-free medium. Since, depending on the species, endothelium-dependent hyperpolarization is mediated by the activation of either K_{Ca} or K_{ATP} channels in vascular smooth muscle, several endothelium-dependent hyperpolarizing factors may exist.

Prostacyclin

Prostacyclin is formed from arachidonic acid following the activation, in turn, of phospholipase A_2 , cyclooxygenase, and prostacyclin synthase. Its release,

which depends mainly upon calcium release from intracellular stores, is observed during activation of endothelial cells by shear stress or different agonists, which also cause the release of NO. In certain cases, endothelium-derived prostacyclin causes endothelium-dependent relaxation of vascular smooth muscle cells by stimulating adenylate cyclase and increasing the level of cyclic AMP, but in most cases prostacyclin has rather weak vasodilator properties (Figure 3).

Other relaxing factors

Carbon monoxide

Carbon monoxide (CO) is synthesized during activation of hemoxygenase-1 and -2 in the blood vessel wall. It may contribute to endothelium-dependent relaxation in blood vessels that are insensitive to NO synthase inhibitors. Since CO augments cyclic GMP levels, the relaxation it causes is sensitive to inhibitors of soluble guanylate cyclase. CO also downregulates the expression of endothelin-1 and growth factors in endothelial cells.

C-type natriuretic peptide

Endothelial cells produce C-type natriuretic peptide (CNP). Exogenous CNP causes relaxation of isolated arteries and veins by activation of particulate guanylate cyclase, and, in some blood vessels, by activation of soluble guanylate cyclase and calcium-dependent K⁺ channels. However, evidence for endothelial CNP-mediated endothelium-dependent relaxation *in vivo* is still awaited. Experiments on cocultures of endothelial and smooth muscle cells suggest that endothelial cells may release sufficient amounts of CNP to negatively control the proliferation of vascular smooth muscle cells.

Parathyroid hormone-related peptide

Parathyroid hormone-related peptide may be synthesized both by endothelial cells and by vascular smooth muscle cells. This peptide induces relaxation of isolated vessels and causes hypotension when infused *in vivo*. As with CNP, a possible role of this peptide in endothelium-dependent regulation of vascular smooth muscle tone has yet to be demonstrated.

RELEASE OF RELAXING FACTORS

Besides acetylcholine, there are a number of physiological stimuli or mechanisms that can cause endothelium-dependent relaxation.

Shear stress

The shear stress exerted by the blood flowing on the arterial wall is one of the main factors in the release of relaxing mediators.^{2,4,16} This conclusion was reached from studies showing that an increase in flow rate through an isolated artery substantially increased the relaxation. A similar result was obtained if a stable flow rate was replaced by a pulsatile one (Figure 5).¹⁷ This effect explains why flow-induced dilatation is endothelium-dependent in the intact organism. Thus, if resistance vessels in a peripheral organ (heart or skeletal muscle) suddenly dilate, the resulting surge of blood causes dilatation of large arteries irrigating that organ. This dilatation is not observed in arteries without endothelium. These flow-induced changes in vessel diameter tend to normalize wall shear stress, making it return to baseline values. The augmented release of endothelium-derived relaxing factors induced by shear stress results—at least in part—from the activation of the kinin-kallikrein system and the local formation of bradykinin in the vessel wall.

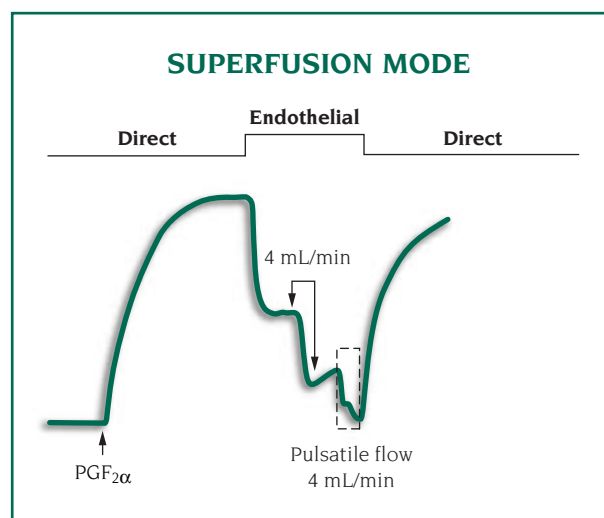


Figure 5. Effect of increase in flow rate under bioassay conditions. A bioassay ring without endothelium (canine coronary artery) is superfused either with the effluent of a stainless tube (direct superfusion) or with the perfusate through a segment of femoral artery with endothelium (endothelial superfusion). This bioassay ring is used to detect the relaxant activity of the perfusate at an initial flow rate of 2 mL/min. The bioassay ring is first contracted with prostaglandin F_{2α} (PGF_{2α}) and then moved under the endothelial superfusion. A partial relaxation is observed in the bioassay ring, due to the production of relaxing substances by the endothelium of the perfused segment. The relaxation is augmented by increasing the flow rate from 2 to 4 mL/min, and pulsatile flow induces a full relaxation of the bioassay ring. The increased release of relaxing factors is best explained by the effect of shear stress on the endothelial cells. Adapted from ref 17: Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol.* 1986;250(6, pt 2):H1145-H1149. Copyright © 1986, American Society for Investigative Pathology. With permission.

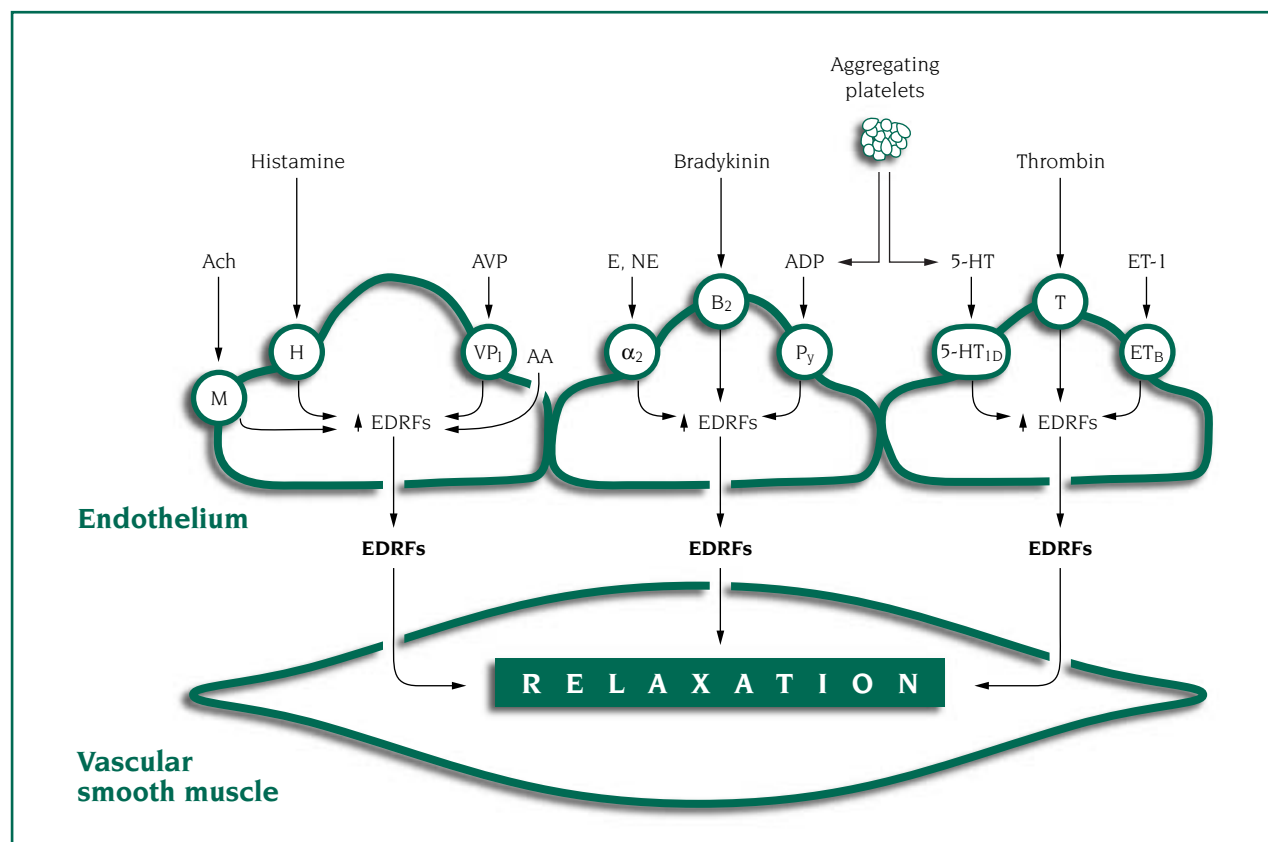


Figure 6. Activation of endothelial receptors and release of endothelium-derived relaxing factors (EDRF), which cause the relaxation of the underlying smooth muscle. AA, arachidonic acid; α_2 , α_2 -adrenoceptor; Ach, acetylcholine; ADP, adenosine diphosphate; AVP, arginine vasopressin; B_2 , bradykinin-2 receptor; E, epinephrine; ET_B , endothelin-B receptor subtype; H, histamine receptor; 5-HT, serotonin, 5-hydroxytryptamine; $5-HT_{1D}$, $5-HT_{1D}$ serotonergic receptor; M, muscarinic receptor; NE, norepinephrine; P_y , purinergic receptor; T, thrombin receptor; VP_1 , vasopressinergic-1 receptor. Reproduced from ref 12: Vanhoutte PM. Endothelial dysfunction and inhibition of converting enzyme. Eur Heart J. 1998;19(suppl J):J7-J15. Copyright © 1998, The European Society of Cardiology. With permission.

Endothelium-dependent relaxation mediated by shear stress involves integrins (transmembrane glycoproteins involved in cell-matrix interactions) and the endothelial cytoskeleton. Indeed, both inhibition of integrin binding to the extracellular matrix and disruption of the endothelial cytoskeleton specifically inhibit flow-induced dilation without affecting endothelium-dependent relaxation to agonists. Shear stress on the endothelial cells can stimulate the immediate release of NO, prostacyclin, and EDHF. In addition, long-term exposure to shear stress upregulates the expression of type III NO synthase in cultured endothelial cells. Therefore, the continuous stimulation of endothelial NO release by flowing blood probably contributes to the protective role of the endothelium against platelet, neutrophil, and monocyte adhesion in the intact organism. In addition, when blood flow is augmented for a sustained period of time, NO participates in the long-term increase in diameter of the vessel.

Activation of endothelial receptors

The endogenous substances stimulating the release of relaxing factors are either circulating hormones, autacoids formed by the blood vessel wall, or substances released during coagulation of the blood (Figure 6).^{2,12,16}

Hormones

Epinephrine (adrenaline), norepinephrine (noradrenaline), and synthetic α_2 -adrenergic agonists can cause endothelium-dependent relaxations, which are prevented by α_2 -adrenergic antagonists, thus demonstrating the presence of α_2 -adrenoreceptors on these endothelial cells. It is likely that endothelium-dependent relaxations participate in the vasodilator effect of catecholamines in some blood vessels (like the coronary arteries). In rat cerebral arterioles, α_2 -adrenergic agonists cause relaxation by augmenting the direct relaxing effect of basally released NO on vascular smooth

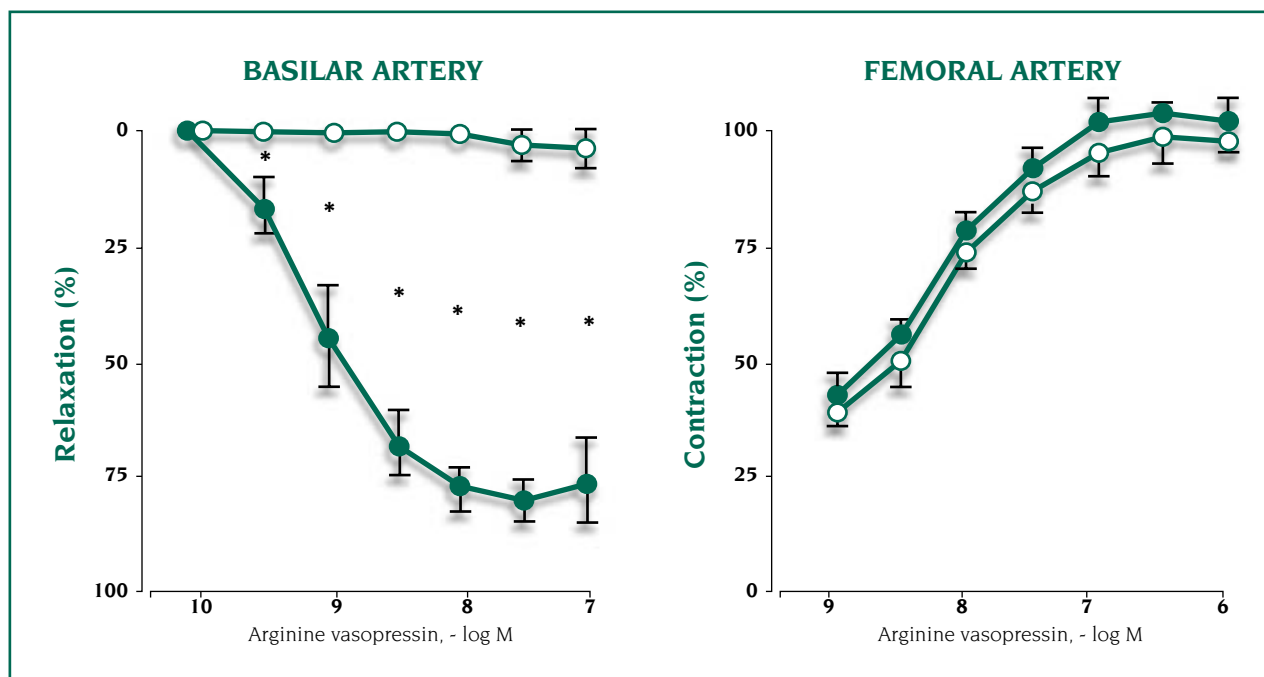


Figure 7. Differential effect of arginine-vasopressin on vascular reactivity in canine cerebral and peripheral arteries.

Left: arginine-vasopressin induces profound relaxation in basilar arteries with endothelium (closed circles), but not in those without endothelium (open circles).

Right: in contrast, arginine vasopressin directly contracts the smooth muscle of the femoral artery without causing the release of endothelial vasoactive factors. *The difference between the two types of preparations is statistically significant ($P < 0.05$). Adapted from ref 18: Katusic ZS, Shepherd JT, Vanhoutte PM. Vasopressin causes endothelin-dependent relaxations of the canine basilar artery. *Circ Res.* 1984;55:575-579. Copyright © 1984, American Heart Association. With permission.

muscle cells and not by augmenting the release of NO from the endothelium. The exact mechanism of the permissive role of NO in these vessels is unknown.

Vasopressin and oxytocin cause endothelium-dependent responses by acting on endothelial V_1 vasopressin-ergic receptors in certain arteries. This effect is particularly striking in cerebral arteries where vasopressin causes potent endothelium-dependent relaxation, while it does not have this effect in the peripheral circulation (Figure 7).¹⁸ The endothelium-dependent dilatation induced by vasopressin may contribute to the preferential redistribution of blood flow to the brain when the hormone is secreted, eg, during hemorrhage.

Autacoids

The autacoids that release EDRF include histamine, bradykinin, and various neuropeptides.

Histamine causes potent endothelium-dependent relaxation in many blood vessels. The endothelial action of histamine in arterioles likely accounts for the local vasodilatation and hence for the rubor characteristic of the histamine response.

Various neuropeptides, in particular substance P, cause endothelium-dependent relaxation. The endothelial effect of substance P may be involved in the local vasodilatation characteristic of antidromal (axon reflex) stimulation of sensory nerves.

Bradykinin is a potent stimulator of the release of EDRF and causes major precapillary vasodilatation, possibly by its action on endothelial B_2 kinin receptors. Bradykinin releases both NO and, at higher concentrations, EDHF, as observed in human coronary arteries (Figure 3). Since the precursors of bradykinin (kininogens) are found throughout the body and particularly in platelets, and given that the vessel wall contains the enzymes required to transform these kininogens into bradykinin, locally generated bradykinin may have a more important role in the regulation of vasomotor tone than was originally thought. Indeed, several observations suggest that the local production of bradykinin mediates endothelium-dependent relaxations to increases in flow. Inhibition of local bradykinin degradation by angiotensin-converting enzyme inhibitors may contribute to the beneficial effect of these compounds, in addition to inhibiting the formation of angiotensin II.



Platelet products and thrombin

The endothelial action of thrombin and of the platelet products 5-hydroxytryptamine (serotonin) and adenosine diphosphate (ADP) is of crucial importance in the protective role of the endothelium against undesired coagulation. Thus, local platelet aggregation, with the inevitable release of ADP and serotonin, as well as the local formation of thrombin, leads to massive endothelium-dependent vasodilatation (Figure 6). This helps to eliminate platelet microaggregates. In addition, NO, in synergy with prostacyclin, also inhibits further platelet adhesion and aggregation, thereby eliminating the danger of vascular occlusion. Conversely, if the endothelial lining is damaged, eg, as a result of trauma, aggregation proceeds, resulting in continuous release of serotonin and thromboxane A₂, which have unrestricted access to the smooth muscle. Hence, the smooth muscle contracts and the blood vessel closes, giving rise to the vascular phase of hemostasis.

ENDOTHELIUM-DEPENDENT CONTRACTION

Endothelial cells also can initiate contraction of the underlying smooth muscle by releasing vasoconstrictor substances. Endothelium-derived contracting factors (EDCFs) include the peptide endothelin, vasoconstrictor prostanoids such as thromboxane A₂ and prostaglandin H₂, as well as superoxide anions and components of the renin-angiotensin system. Whereas the release of NO and other relaxing factors appears to be decreased in many vascular diseases, the release of EDCFs is, by contrast, augmented.^{2,16}

Contractions blocked by cyclooxygenase inhibitors

A group of EDCFs is generated by the metabolism of arachidonic acid through the cyclooxygenase pathway.² As shown in Figure 8, particularly in peripheral veins, but also in the cerebral circulation and in some

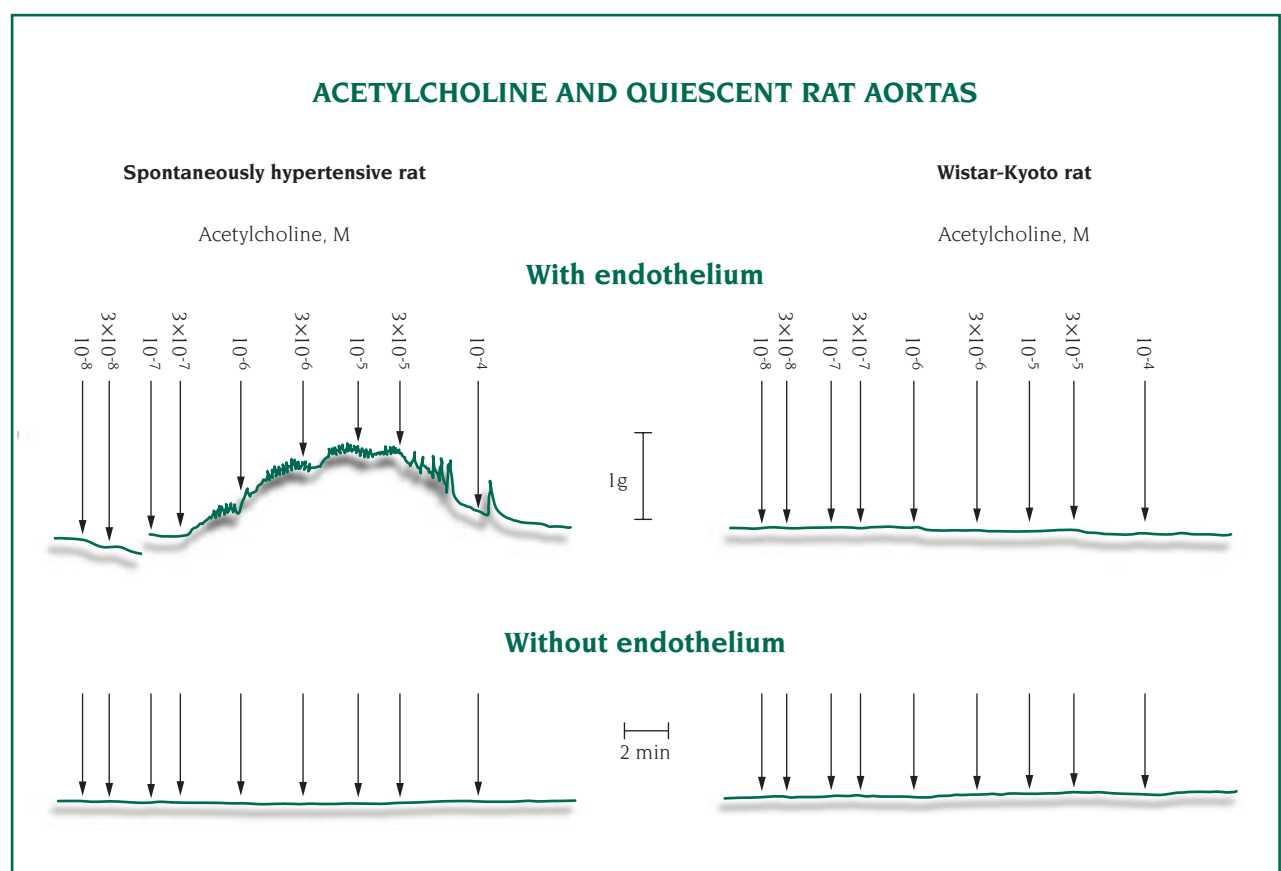


Figure 8. Isometric tension changes in quiescent rings of rat aorta with (above) and without (below) endothelium, from normotensive and spontaneously hypertensive animals (right and left, respectively). Acetylcholine induces endothelium-dependent contractions only in aortas from the spontaneously hypertensive rats. Based on data from ref 20.

arteries from hypertensive animals, endothelium-dependent contractions are mediated by thromboxane A_2 or prostaglandin H_2 , which activate the same thromboxane-endoperoxide receptor.^{16,19,20}

These mediators may be involved in the autoregulation of the cerebral circulation. Few physiological stimuli cause endothelium-dependent contractions that are sensitive to inhibitors of cyclooxygenase. An important response is probably that to stretch (*Figure 9*).²¹ Thus, endothelium-dependent contraction of a cerebral artery in response to stretch closely resembles the autoregulatory response. A possible explanation for the autoregulation of the cerebral circulation initiated by a sudden stretch of the vessel wall in response to an increase in blood pressure is the release of endothelium-derived contracting factors, which would activate the underlying smooth muscle to restore a normal flow rate.

In addition, the cyclooxygenase pathway is a source of superoxide anions, which can cause contraction directly or indirectly by inactivating EDRF-NO. Endothelial cells also produce reactive oxygen species

either through the xanthine oxidase and NADPH-oxidase systems, or the NO synthases when available concentrations of tetrahydrobiopterin or L-arginine are too low.

Hypoxia-induced contraction

Some blood vessels, particularly coronary, cerebral, and pulmonary arteries, rapidly contract when exposed to sudden hypoxia. This endothelium-dependent contraction is caused by the transfer of a diffusible substance, which is still unknown. It is not dependent on the cyclooxygenase pathway nor on the release of endothelin. The phenomenon is exacerbated by a reduced release of NO.

Endothelin

Although endothelin exists in three isoforms (endothelin-1, -2, and -3), endothelial cells exclusively produce endothelin-1.²² Translation of the mRNA generates preproendothelin, which is converted to big endothelin; its conversion to the mature peptide endothelin-1 by endothelin-converting enzymes is necessary for the development of its vascular activity. The expression of mRNA and the release of the peptide from cultured endothelial cells is stimulated by thrombin, transforming growth factor β_1 , interleukin-1, epinephrine, angiotensin II, arginine vasopressin, calcium ionophore, and phorbol ester. Endothelin-1 causes vasodilation at lower concentrations by activating endothelial ET_B receptors coupled to the release of NO, prostacyclin, and EDHF, while at higher concentrations it causes marked and sustained contractions by activation of ET_A , and, in some blood vessels, of ET_B receptors on vascular smooth muscle.^{22,23} Circulating levels of endothelin-1 are low, suggesting either discrete endogenous production under physiological conditions, the presence of potent inhibitory mechanisms (such as the negative control induced by NO), or preferential abluminal release of the peptide towards vascular smooth muscle cells.

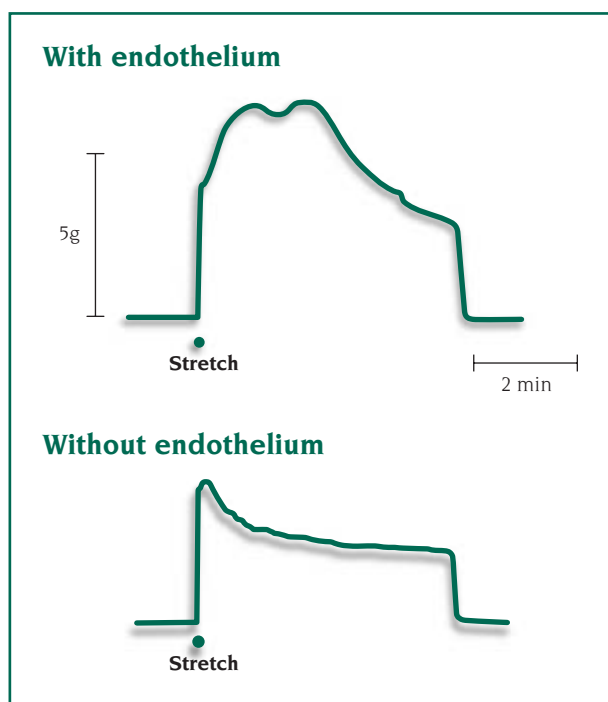


Figure 9. Isometric tension response of canine basilar artery rings with (above) and without (below) endothelium to sudden stretch. In the rings without endothelium, the rapid increase in tension in response to stretch is followed by a slow decrease in tension (hysteresis). In the rings with endothelium, on the other hand, stretch induces a contraction which is stable for approximately 2 minutes. This experiment shows that stretch causes endothelium-dependent contractions in the canine basilar artery with a time course comparable to that of the autoregulatory response. Based on data from ref 21.

ENDOTHELIUM-DERIVED FACTORS AND PATHOLOGY

Regenerated endothelium

In a healthy adult body, under normal conditions (and in the absence of physiological local angiogenesis as observed in women), endothelial cells proliferate at a very low rate. This rate of proliferation increases with aging, after angioplasty, or under pathological conditions

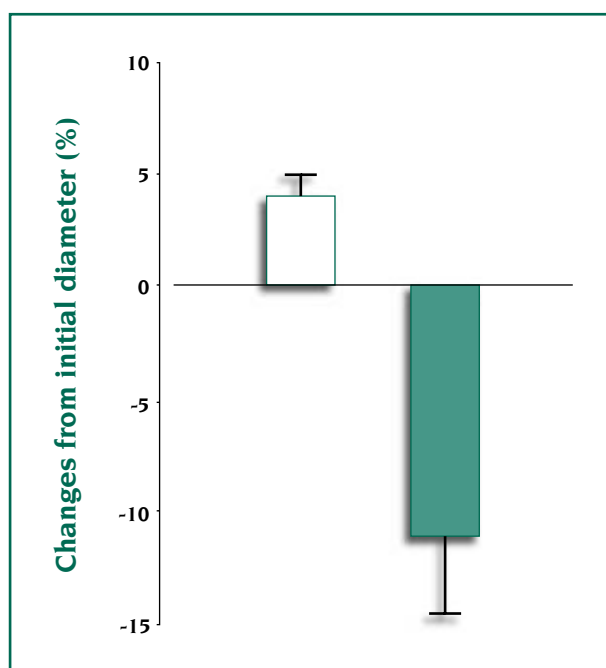


Figure 10. Changes in porcine coronary artery diameter following intracoronary infusion of serotonin (10 mg/kg). In control coronary arteries (open bar), serotonin causes a slight increase in diameter, while in coronary arteries with regenerated endothelium (hatched bar), serotonin decreases the diameter. C.M. Boulanger, unpublished data.

such as hypertension. However, the newly generated endothelial cells appear to be dysfunctional.²⁴ In the model of regenerated endothelium following balloon abrasion of the porcine coronary artery, a selective dysfunctional release of relaxing factors is observed in response to agonists activating receptors linked to pertussis toxin-sensitive Gi proteins (such as serotonin 5-HT_{1D}, endothelin ET_B, and α_2 -adrenergic receptors). However, the response of regenerated endothelial cells to agonists activating receptors not coupled to pertussis toxin-sensitive Gi proteins is maintained (eg, response to bradykinin or ADP).¹⁶ These dysfunctional endothelial cells can promote the occurrence of coronary vasospasm in response to ergonovine or platelet-derived products such as serotonin, by leaving unopposed the direct contractile effect of the serotonergic agonist on vascular smooth muscle cells (Figure 10). This vasospastic event is associated with signs of severe cardiac ischemia. In porcine coronary arteries, this selective dysfunction of regenerated endothelial cells is still observed 5 to 6 months after the abrasion. Administration of growth factors (such as fibroblast growth factor or vascular endothelial cell growth factor) may accelerate the regrowth of the endothelium, but no information is available regarding a possible beneficial effect of these growth factors

on endothelial dysfunction. It has been also shown that vessels growing de novo in the intact organism, as in collateral formation or tumor angiogenesis, are hypersensitive to the vasoconstrictor effect of serotonin; this can also be explained by the absence of an appropriate endothelium-dependent response to serotonin.

The fact that the response to all endothelial receptors coupled to pertussis toxin-sensitive Gi proteins is selectively altered has suggested that these Gi proteins may be dysfunctional in regenerated endothelial cells. Regenerated endothelial cells express a normal amount of α -subunits of Gi proteins as compared with normal endothelial cells, but their function may be altered. Interestingly, Gi proteins are preferentially expressed in endothelial cells from large human epicardial coronary arteries in contrast to those from small coronary arterioles, which are less prone to the occurrence of atherosclerosis.

Reperfusion injury

A situation similar to that of the dysfunction of regenerated endothelial cells occurs during abrupt reperfusion. When the blood flow is restored following temporary occlusion of a coronary artery, the reperfusion injury involves not only the myocardium, but also the blood vessel wall. In canine coronary arteries, 1 hour after reperfusion, almost all endothelium-dependent responses have disappeared or are severely inhibited. This is not surprising, as reperfusion involves profound metabolic changes due to the reintroduction of oxygen and massive free radical formation. A greater cause for concern is that 3 months after reperfusion injury, the reactivity of the reperfused artery is still abnormal, particularly with regard to the response of platelet aggregation and thrombin. As a result, hyperconstriction is observed and platelets and other circulating cells adhere to the reperfused segment. This abnormal adhesion can lead to the release of vasoconstrictors or growth factors from adherent cells, or favor the transmigration of monocytes to the subendothelium, initiating a local inflammatory response. If the reperfusion is carried out slowly, the subsequent chronic endothelial dysfunction may not be so dramatic.

Atherosclerosis

A significant decrease in endothelium-dependent relaxation has been observed in various models of atherosclerosis as well as in human atherosclerotic coronary arteries. As with regenerated endothelial cells,

the first endothelium-dependent response to be lost is that to serotonin.^{16,24} The most important mechanism in the decrease in endothelium-dependent relaxation appears to be a reduced release of NO.¹³ As the disease progresses over the years and the artery thickens and stiffens, it becomes increasingly difficult for NO to reach the vascular smooth muscle before it is inactivated. Animal models of atherosclerosis have evidenced the presence of an inducible NO synthase expressed in the vessel wall, although the amount of active NO produced is significantly impaired due to an increased formation of superoxide anions.^{13,25} The beneficial effect of chronic treatment with L-arginine in some animal models of atherosclerosis may be explained by an increased availability of the substrate of both isoforms, resulting in a greater generation of NO, although a decreased formation of superoxide anions certainly plays a role as well. Inhibition of superoxide anions in the atherosclerotic wall may also explain the beneficial effect of chronic treatment with antioxidants. An augmented expression of endothelin-1 has been observed in the human atherosclerotic plaque, but is unlikely to directly contribute to the impaired endothelium-dependent vasodilation, since endothelin receptor antagonists do not improve endothelium-dependent responses.

It is tempting to hypothesize that endothelial dysfunction is one of the initial steps involved in the development of atherosclerosis. As endothelial cells regenerate with aging or following exposure to risk factors such as hypertension, smoking, or hyperlipidemia, their protective role against platelet, monocyte, or neutrophil adhesion diminishes. This may set the stage for the development of an inflammatory response, which, together with the presence of oxidized low-density lipoprotein in the vessel wall, further worsens the initial endothelial dysfunction.

Hypertension

In most animal models of hypertension, the endothelium-dependent relaxation to acetylcholine (and other agonists) is impaired.^{16,19} However, this endothelial dysfunction presents different characteristics depending on the model studied. The impairment of endothelial function is either associated with a decreased production of NO and/or a concomitant release of endothelial contracting factors that impair the effects of NO. This may explain the apparent discrepancy between the results from studies evaluating endothelial function and the contribution of NO in hypertensive subjects. Indeed, endothelium-dependent dilatation is either decreased or unchanged in patients with high blood

pressure. However, most studies suggest a dysfunctional release or effect of EDRF in hypertensive vessels.

In salt-sensitive hypertensive Dahl rats, endothelium-dependent relaxation is decreased as a result of impaired release of NO associated with decreased activity of the calcium-dependent NO synthase in blood vessels and increased levels of the endogenous inhibitor ADMA. In this model, exposure to L-arginine restores endothelium-dependent relaxation. In conduit arteries of spontaneously hypertensive rats (genetic hypertension), endothelium-dependent relaxation to acetylcholine, ADP, or serotonin is impaired due to the release of endoperoxides, in particular prostaglandin H₂, which activate thromboxane-endoperoxide receptors.¹⁹ Endothelial function is normalized following inhibition of cyclooxygenase activity or blockade of thromboxane-endoperoxide receptors. The endothelial dysfunction associated with activation of thromboxane-endoperoxide receptors may result from impaired NO action on smooth muscle cells or altered endothelial NO release. Calcium-dependent NO activity is increased in homogenates from spontaneously hypertensive rat (SHR) blood vessels, suggesting either an increased expression of endothelial NO synthase or the presence of another isoform. The presence of a cyclooxygenase-dependent EDCF in hypertension is supported by the fact that a cyclooxygenase inhibitor (indomethacin) improves the endothelium-dependent response to acetylcholine in patients with essential hypertension. In the microcirculation of the SHR, endothelium-dependent relaxation to acetylcholine appears to be decreased as a result of the concomitant release of superoxide anions.

Endothelial dysfunction in hypertension appears to be a consequence of high blood pressure, since the majority of antihypertensive treatments normalize these responses. However, it may also amplify the increase in vascular resistance, since the inhibition of NO release (as in type III NO synthase knockouts or after enzyme inhibition by L-arginine analogs) causes an increase in blood pressure.

Heart failure

Heart failure is a clinical syndrome that occurs late in the course of coronary artery disease and other vascular diseases. It is accompanied by a generalized increase in peripheral vascular resistance, which may result from compensatory mechanisms involving neural, hormonal, or local factors. Endothelial cell dysfunction may also contribute to the peripheral vasoconstriction.²⁶ Indeed, decreased endothelium-dependent relaxation has been reported in several experi-



mental models of heart failure. Likewise, in patients with heart failure, the vasodilator response to acetylcholine is impaired. Endothelial dysfunction may be specific to certain responses (eg, acetylcholine), but has not been observed with other endothelium-dependent vasodilators (eg, substance P, the calcium ionophore A23187). In addition, plasma levels of endothelin-1 increase in proportion to the severity of cardiac failure. Chronic treatment with a combined ET_A-ET_B endothelin antagonist significantly improves long-term survival in rat models of heart failure.

Cerebral vasospasm

Experimental cerebral vasospasm is associated with a disappearance or dramatic decrease in endothelium-dependent relaxation. However, the release of endothelium-derived NO appears to be normal as evidenced from bioassay experiments and measurement of cyclic GMP. The abnormality in endothelium-dependent relaxation involves a decreased response of vascular smooth muscle to endothelial NO. This appears logical, since the cerebral vasospasm that normally follows subarachnoid hemorrhage is associated with the presence in the tissues of hemoglobin, a scavenger of NO. In addition, the potent vasoconstrictor endothelin-1 may play a role in the spastic response, since endothelin-1 levels are increased in cerebrospinal fluid and endothelin antagonists normalize the response in subarachnoid hemorrhage.

THREE KEY QUESTIONS

In the following section of this issue, three experts will focus on some of the points raised in this paper. Michel Félétou and Paul Vanhoutte reply to the question of which factors are involved in endothelium-dependent relaxation: **“is nitric oxide the only answer?”** and clearly show that NO should not be viewed as eclipsing the role of other candidates. Endothelin is known to contribute to the pathogenesis of a number of cardiovascular diseases, and so Thomas Lüscher and Francesco Cosentino ask **“does endothelin play a role in hypertension?”** and the corollary, what is the potential for endothelin receptor antagonists to become part of the therapeutic arsenal against hypertension? But however exciting future drugs may appear, those that seem familiar to us may still hold a few surprises: Helmut Drexler, in his contribution entitled **“does bradykinin play a role in the regulation of vascular tone?”** illustrates this point by showing that part of the beneficial effect of ACE inhibition may be attributable to an improvement in endothelial function via an increase in bradykinin availability.

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