The endothelial cells have an obligatory role in the relaxation of isolated arteries in response to acetylcholine! This simple but seminal observation has brought recognition of the pivotal role of the endothelium in contributing to the physiological regulation of vasomotor control. This endothelium-dependent control is exerted by the release of several diffusible substances [endothelium-derived relaxing (EDRF) and contracting (EDCF) factors] from the endothelial cells. This essay summarizes briefly the observations obtained mainly in the author's laboratory that have determined how the secretion by endothelial cells of the relaxing factors underlies moment-to-moment changes in the tone of the surrounding vascular smooth muscle cells, and how the inability of the endothelial cells to do so eventually initiates atherosclerosis and thus vascular disease. For detailed references, the reader is referred to several more exhaustive overviews.2–13

Endothelium-Derived Relaxing Factors (Fig 1)

Endothelium-Derived Nitric Oxide

The short-lived diffusible, nonprostanoid substance that mediates the endothelium-dependent relaxation to acetylcholine originally described by Furchgott and Zawadzki\(^1\) has been identified as nitric oxide (NO). NO is formed in endothelial cells from the guanidine-nitrogen terminal of \(\text{L-arginine}\), by the constitutive NO-synthase III (endothelial NO-synthase, eNOS). The activation of NO synthase III depends on the intracellular concentration of calcium ions in the endothelial cells, as it is \(\text{Ca}^{2+}\) – calmodulin-dependent. The activity of the enzyme requires cofactors such as reduced nicotinamide-adenine-dinucleotide phosphate, and 5,6,7,8 tetra-hydrobiopterin. Endothelial NO-synthase can be inhibited competitively by \(\text{L-arginine analogs such as NG-monomethyl-L-arginine or N\text{G}-nitro-L-arginine}\). NO diffuses to the vascular smooth muscle cells and relaxes them mainly by stimulating the cytosolic enzyme, soluble guanylate cyclase, which catalyzes the production of cyclic 3'5'-guanosine monophosphate (cGMP), leading to inhibition of the contractile process. NO is a major contributor to endothelium-dependent relaxation in large arteries, including the coronary, systemic, mesenteric, pulmonary and cerebral arteries. In vivo, inhibitors of NO synthase cause vasoconstriction in most vascular beds and an increase in systemic arterial pressure in both animals and humans.\(^2–14\)

NO is released not only towards the underlying vascular smooth muscle, but also into the lumen of the blood vessel. Thus, at the interface between the blood and the vascular wall, NO inhibits the adhesion of platelets and white cells to the endothelium. It acts (in strong synergy with prostacyclin) to inhibit platelet aggregation.\(^3,9,14\) It also inhibits the growth of the vascular smooth muscle cells and prevents the production of adhesion molecules and endothelin (Fig 2).\(^1,5\)

The production of NO is regulated by physical and humoral stimuli. Thus, the shear stress exerted by the flowing blood on the endothelial cells is one of the main factors determining the local release of NO. This underlies flow-dependent vasodilatation. Several hormones and autacoids

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Endothelial Control of Vasomotor Function
— From Health to Coronary Disease —

Paul M. Vanhoutte
CONTROL ENDOTHELIAL CELL

**Inhibition of:**

1. Smooth muscle contraction
2. Platelet aggregation
3. Smooth muscle proliferation
4. Monocyte and platelet adhesion
5. Expression adhesion molecules
6. Oxidation of LDL
7. Endothelin production

**EDRF-NO**

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**Fig 2.** Postulated signal transduction processes in a normal endothelial cell. Activation of the cell causes the release of EDRF–NO, which has important protective effects in the vascular wall. \( \alpha \)-alpha-adrenergic; 5-HT, serotonin-receptor; ET, endothelin receptors; B, bradykinin receptor; P, purinoceptor; G, coupling proteins; cAMP, cyclic adenosine monophosphate; NO, nitric oxide; LDL, low density lipoproteins. (From Vanhoutte PM. Endothelial dysfunction and vascular disease. In: Panza JA and Cannon RO III, editors. Armonk, NY: Futura Publishing; 1999: by permission).

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**Fig 3.** Some of the neurohumoral mediators that cause the release of endothelium-derived relaxing factors (EDRF) through activation of specific endothelial receptors (circles). A, adrenaline (epinephrine); AA, arachidonic acid; Ach, acetylcholine; ADP, adenosine diphosphate; \( \Pi \)-\( \alpha \)-alpha adrenergic receptor; AVP, arginine vasopressin; B, bradykinin receptor; ET, endothelin, endothelin-receptor; H, histaminergic receptor; 5-HT, serotonin (5-hydroxytryptamine), serotoninergic receptor; M, muscarinic receptor; NA, noradrenaline (norepinephrine); P, purinergic receptor; T, thrombin receptor; VP, vasopressinergic receptor. (From Vanhoutte PM. Endothelial dysfunction and vascular disease. In: Panza JA and Cannon RO III, editors. Armonk, NY: Futura Publishing; 1999: by permission).

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**Fig 4.** Interaction between platelet products, thrombin and endothelium. If the endothelium is intact, several of the substances released from the platelets [in particular, the adenine nucleotides (ADP and ATP) and serotonin (5-HT)] cause the release of endothelium-derived relaxing factors (EDRF) and prostacyclin (PGI2). The same is true for any thrombin formed. The released EDRF will relax the underlying vascular smooth muscle, opening up the blood vessel, and thus flushing the microaggregate away; it will also be released towards the lumen of the blood vessel to prevent platelet adhesion to the endothelium and, synergistically with prostacyclin, inhibit platelet aggregation. In addition, monoamine oxidase (MAO) and other enzymes will break down the vasoconstrictor serotonin, limiting the amount of the monoamine that can diffuse toward the smooth muscle. Finally, the endothelium acts as a physical barrier that prevents access to the smooth muscle of the vasoconstrictor platelet products, serotonin and thromboxane A2 (TXA2). These different functions of the endothelium play a key role in preventing unwanted coagulation and vasospastic episodes in blood vessels with a normal intima. If the endothelial cells are removed (eg, by trauma), the protective role of the endothelium is lost locally, platelets can adhere and aggregate, and vasoconstriction follows; this contributes to the vascular phase of hemostasis. +, activation; –, inhibition. (From Vanhoutte PM. Endothelial dysfunction and vascular disease. In: Panza JA and Cannon RO III, editors. Armonk, NY: Futura Publishing; 1999: by permission).
curtailed. Aggregation proceeds with the continuous release of serotonin and thromboxane A2, both of which contract the underlying vascular smooth muscle with resulting closure of the blood vessel leading to the vascular phase of hemostasis (Fig 4).3,8,13

Prostacyclin

Prostacyclin, a product of cyclooxygenase, is formed primarily in endothelial cells and causes relaxation of certain vascular smooth muscle by activating adenylate cyclase and increasing the production of cyclic 3',5'-adenosine monophosphate (cAMP). In most blood vessels the contribution of prostacyclin to endothelium-dependent relaxation is not major, to judge from the limited effect of cyclooxygenase-inhibitors on these responses. However, prostacyclin acts synergistically with NO to inhibit platelet aggregation (Fig 4).

Endothelium-Dependent Hyperpolarizing Factor

In various animal and human arteries, acetylcholine, and other endothelium-dependent vasodilators, cause endothelium-dependent hyperpolarizations that contribute to endothelium-dependent relaxations. These hyperpolarizations have been attributed to a diffusible endothelium-derived hyperpolarizing factor (EDHF), different from NO and prostacyclin, although the latter two can hyperpolarize certain vascular smooth muscle cells. The exact nature of EDHF remains speculative. Among the more recent candidates to explain endothelium-dependent hyperpolarizations, gap junction, epoxyeicosatrienoic acids (EETs), potassium ions and hydrogen peroxide are the major contenders (Fig 1).17–19

The contribution of hyperpolarization to endothelium-dependent relaxation varies as a function of the size of the artery20 and thus is more pronounced in resistance vessels. In large arteries, both mediators can contribute to endothelium-dependent relaxation, but the role of NO predominates under normal circumstances. However, in the large arteries, EDHF can mediate near normal endothelium-dependent relaxation when the synthesis of NO is inhibited or dysfunctional. In certain cases, NO exerts an inhibitory effect on endothelium-dependent hyperpolarizations.21

Chronic Modulation

Several chronic modulatory influences can upregulate the release of relaxing factors by endothelial cells, including chronic exposure to estrogens, chronic increases in blood flow, exercise training and intake of ω-unsaturated fatty acids, red wine polyphenols or other antioxidants.22–25

Endothelial Dysfunction

In the course of ageing, and in several types of vascular disease and hypertension, the endothelial cells become dysfunctional.3,8,10–13 which is evidenced by an impairment in endothelium-dependent relaxation, caused mainly by a reduced release of EDRFs, in particular NO, although production of endothelium-derived vasoconstrictor substances may also contribute.2,3,5,26

Regenerated Endothelium

The normal aging process induces a turn-over (apoptotic death, resulting in desquamation followed by regeneration) of endothelial cells resulting in abnormal function. Indeed, regenerated endothelial cells lose some of their ability to release NO, particularly in response to platelet aggregation and thrombin.28,29 This reflects the fact that regenerated endothelial cells respond abnormally to serotonin, and other substances using the pertussis toxin-sensitive pathway controlling the release of NO (Fig 2). In such regenerated endothelial cells, the pertussis toxin sensitive Gi proteins are present, but exhibit a reduced activity. The loss of the pertussis toxin-sensitive response is selective and does not apply, at least initially, to endothelium-dependent responses mediated by Gq-coupling proteins. It may be caused by the greater accumulation of oxidized low-density lipoproteins (LDL) by the regenerated endothelial cells. The reduced release of NO can be compensated in part by the larger contribution of EDHF to endothelium-dependent relaxation.28–32

Hypercholesterolemia and Atherosclerosis

Hypercholesterolemia induced by high-fat and/or high-cholesterol diets impairs endothelium-dependent relaxation.33,34 By contrast, endothelium-independent relaxation in response to nitroglycerin or sodium nitroprusside remains largely normal. To judge from animal data, in the early stage of the atherosclerotic process, endothelial dysfunction is limited to the pertussis toxin-sensitive, Gi protein-dependent pathway (Fig 2). Thus, the regenerated endothelial cells, chronically exposed to high concentrations of cholesterol, have a reduced ability to ADP-ribosylate the pertussis toxin.35 Consequently, in coronary arteries from hypercholesterolemic pigs, endothelium-dependent relaxations evoked by agents that activate the pertussis toxin-sensitive Gi protein (eg, serotonin, ω-adrenergic agonists, aggregating platelets, thrombin) are depressed whereas those induced by ADP, bradykinin, are better preserved.16,28,29,33–35

Oxidized LDLs induce, in vitro, a similar selective endothelial dysfunction, but at higher concentrations they also inhibit endothelium-dependent responses evoked by other stimuli.36

The most important mechanism in the reduction of the endothelium-dependent response is decreased release or bioavailability of NO. Endothelial dysfunction is probably a fundamental initial step in the progression of atherosclerosis. This hypothesis implies that ageing and prolonged exposure to shear stress, coupled with risk factors such as obesity, diabetes, hypertension, and smoking, accelerate the endothelial turn-over and hence the process of endothelial regeneration. As a result, larger and larger sections of the endothelium (particularly in the areas of turbulence) become unable to resist platelet adhesion and aggregation and respond less well to thrombin formation. The feed-back effect of NO (together with prostacyclin) on platelet aggregation decreases steadily, while vasoconstrictor factors (serotonin and thromboxane A2) are released in increasingly greater amounts, together with growth factors [such as platelet-derived growth factor (PDGF)], which probably are responsible for initiating the characteristic morphological changes in atherosclerosis, in particular, because the local shortage of NO unleashes the growth process.3,7–13

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