Endothelial dysfunction and atherosclerosis

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The endothelium mediates a number of responses (relaxation or contraction) of arteries and veins from animals and humans. The endothelium-dependent relaxations are due to the release, by endothelial cells, of potent non-prostanoid vasodilator substances. Among these, the best characterized is endothelium-derived relaxing factor (EDRF), which is believed to be nitric oxide (NO). Nitric oxide is formed by the metabolism of l-arginine by the constitutive NO synthase of endothelial cells. In arterial smooth muscle, the relaxation evoked by EDRF is explained by the stimulation by NO of soluble guanylate cyclase that leads to the accumulation of cGMP. In a number of animal blood vessels and in human coronary arteries, the endothelial cells release a substance that causes hyperpolarization of the cell membrane (endothelium-derived hyperpolarizing factor, EDHF). The release of EDRF from the endothelium can be mediated by both pertussis toxin-sensitive (α2-adrenoceptor activation, serotonin, aggregating platelets, leukotrienes) and insensitive (adenosine diphosphate (ADP), bradykinin) G proteins. In blood vessels from animals with regenerated and reperfused endothelium, and/or atherosclerosis, there is a selective loss of the pertussis toxin-sensitive mechanism of EDRF release, which favours the occurrence of vasospasm, thrombosis and cellular growth. The available information from isolated human blood vessels or obtained in situ concurs with the conclusions reached from studies with isolated animal tissues. In addition to relaxing factors, the endothelial cells can produce contracting factors (endothelium-derived contracting factors; EDCFs) which include superoxide anions, endoperoxides, thromboxane A2 and endothelin. From animal studies it can be concluded that the propensity to release EDCFs is maintained, or even augmented, in diseased blood vessels. The switch from a normally predominant release of EDRFs to that of EDCFs may play a crucial role in atherosclerosis.

Key Words: Atherosclerosis, endothelial dysfunction, EDRF, EDHF, EDCFs, G proteins.

Introduction

The normal endothelium contributes to the local regulation of vasomotor tone and the maintenance of a non-thrombogenic surface, acts as a selective barrier controlling permeability and transport of solutes and macromolecules to help metabolize many factors circulating in the blood or generated locally, and helps to control the proliferation of underlying vascular smooth muscle cells and to regulate the adhesion and extravasation of neutrophils, monocytes, and lymphocytes. These properties are due to the ability of endothelial cells to sense humoral and haemodynamic stimuli. Three basic mechanisms underlie these properties: the secretion of endothelium-derived factors; the expression at the cell membrane surface of binding proteins, adhesive molecules, and metabolizing enzymes (converting enzymes); and shape changes. The discovery by Furchgott and Zawadzki of the role played by the endothelial cells in relaxation of isolated arteries in response to acetylcholine initiated a major scientific inquiry into the pivotal role of the endothelium in contributing to the normal physiological function of the vascular wall. It soon became obvious that endothelium-dependent responses are mediated by the release of several diffusible substances (endothelium-derived relaxing factor (EDRF) and endothelium-derived contracting factor (EDCF)) from the endothelial cells. Under normal conditions, relaxing factors, especially nitric oxide (NO), are predominantly released by endothelial cells. This review focuses on how the secretion by endothelial cells of vasoactive substances controls the changes in the tone of the underlying vascular smooth muscle cells and on why dysfunction of the endothelial cells may underlie or accompany atherosclerotic processes. It updates previous overviews.

Endothelium-derived vasoactive factors

Endothelium-derived nitric oxide

Nature, synthesis and release

The labile diffusible (half-life of a few seconds), non-prostanoid substance that mediates the endothelium-dependent relaxation to acetylcholine described by
Release of endothelium-derived factors. Endothelial receptor (R) activation induces an influx of Ca\(^{2+}\) into the cytoplasm of the endothelial cell. When agonists activate the endothelial cells, an increase in inositol phosphate (IP\(_3\)) may contribute to the increase in cytoplasmic Ca\(^{2+}\) by releasing it from the sarcoplasmic reticulum (SR). Following interaction with calmodulin, Ca\(^{2+}\) activates nitric oxide synthase (NOS) and leads to the release of endothelium-derived hyperpolarizing factor (EDHF). The increased Ca\(^{2+}\) also accelerates the formation of prostacyclin (PGI\(_2\)) from arachidonic acid (AA) by cyclooxygenase. NO causes relaxation by activating the formation of cGMP from GTP. Prostacyclin causes relaxation by activating the formation of cAMP from ATP. EDHF causes hyperpolarization and relaxation by opening K\(^+\) channels. Any increase in cytosolic Ca\(^{2+}\) (including that induced by the ionophore A23187) causes the release of relaxing factors. (Reproduced from Boulanger and Vanhoutte and modified from Vanhoutte et al.)

Furchgott and Zawadzki, has been identified as free radical NO. Nitric oxide is formed from the guanidine-nitrogen terminal of l-arginine by an enzyme called NO synthase, which is constitutive in normal endothelial cells (NO synthase III). The activation of this NO synthase depends on the intracellular concentration of calcium ions (Ca\(^{2+}\)) in the endothelial cells, is calmodulin-dependent and requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 5,6,7,8-tetrahydrobiopterin (BH4) for optimal activity. Nevertheless NO can also be produced without an increase in intracellular Ca\(^{2+}\), and shear stress may modulate the level of NO through phosphorylation of NO synthase. The enzyme can be inhibited competitively by l-arginine analogues, such as N\(^{\text{G}}\)-monomethyl-l-arginine or N\(^{\text{G}}\)-nitro-l-arginine.
mesenteric, pulmonary and cerebral arteries. Its significance in vivo is suggested by the observations that inhibitors of NO synthase cause vasoconstriction in most vascular beds and an increase in systemic arterial pressure, both in animals and humans[13,16].

The endothelial cell secretes NO not only toward the underlying vascular smooth muscle but also in the lumen of the blood vessel. Under normal conditions, the presence of oxyhaemoglobin in the erythrocytes immediately neutralizes NO, which only has a physiological role at the interface between the endothelial cells and the blood content. Thus, NO inhibits the adhesion of platelets and leukocytes to the endothelium. It acts (synergistically with prostacyclin) to inhibit platelet aggregation[14-8,16-26,31]. NO also inhibits the growth of the vascular smooth muscle cells[27,28].

The release of NO is modulated by physical and humoral stimuli. Among the physical stimuli, the shear stress exerted by the blood on the arterial wall is one of the main factors regulating the local release of NO. Indeed, flow-induced vasodilatation is endothelium-dependent in vivo[30]. Bioassay studies show that an increase in flow, or the introduction of pulsatile flow, stimulate the release of NO and prostacyclin from the endothelium of the perfused arteries[30]. Vasoconstriction of perfused arteries induced by the elevation of intraluminal pressure can be prevented by removal of the endothelium and is, in part, dependent on a reduced release of NO[31].

**G proteins and endothelium-dependent release of NO**

Several neuronal and humoral mediators cause the release of NO through activation of specific endothelial receptors (Fig. 2). The endogenous substances stimulating this release are either circulating hormones (catecholamines, vasopressin), autacoids generated within the vascular wall (bradykinin, histamine) or mediators released by platelets (serotonin, adenosine diphosphate; ADP) or formed during coagulation (thrombin). The receptors for these substances are coupled to the production of NO (NO synthase) by different G proteins (guanine nucleotide-binding proteins) (Fig. 3). In isolated porcine coronary arteries, direct activation of G proteins evokes endothelium-dependent relaxations, mediated by the release of NO, which are blocked by inhibitors of NO synthase. Pertussis toxin (ADP-ribosylates) has revealed two different pathways for endothelium-dependent relaxations mediated by NO: toxin-sensitive (Gα) and insensitive (Gβγ)[33]. In porcine endothelial cells, α2-adrenoceptors, serotonin receptors and thrombin receptors are coupled to pertussis toxin-sensitive Gi proteins, whereas ADP or bradykinin receptors mediate the production of NO by activation of pertussis toxin-insensitive Gβγ protein[34,36].

Pertussis toxin-sensitive endothelium-dependent relaxations in porcine endothelial cells, involve several α subunits identified as Gαi and Gαi-Gαi3[37] and the major α subunit involved in the release of NO is Gαi2[38]. Gi proteins may activate KCa channels[39]. In isolated porcine blood vessels, the production of NO through the G protein pathway may be related to a tyrosine kinase pathway[40].

Pertussis toxin-insensitive endothelium-dependent relaxations evoked by activation of bradykinin receptors are coupled either to small G proteins (more likely to be involved in the release of NO than...
endothelium-derived hypopolarizing factors EDHF), or by $G_{q}$ proteins.

In addition, using cultured endothelial cells, it is possible to distinguish between a $G$ protein-dependent transient production of NO, in response to a rapid change in flow, and a $G$ protein-independent production of NO, in response to a smooth transition in shear stress.

**NO and platelet aggregation**

From the physiological point of view, the substances produced during platelet aggregation are important releasers of NO. This conclusion is based on the findings that in various species, including humans, platelet aggregation induces endothelium-dependent relaxations and that the presence of endothelial cells substantially inhibits the vasoconstriction induced by thromboxane A2 and platelet-derived serotonin. There are two major mediators of the endothelial response to platelets: serotonin and ADP, which act on 5-HT1D receptors and P2Y receptors, respectively (Fig. 3). The endothelial action of thrombin and platelet products is crucial for the protective role played by the normal endothelium against unwanted coagulation (Fig. 4). Therefore, local platelet aggregation, with the associated release of serotonin and ADP, as well as the production of thrombin (because of the local activation of the coagulation cascade), leads to a major local release of NO, which diffuses toward the underlying vascular smooth muscle, induces its relaxation and thus dilatation of the artery. This reaction helps to eliminate the micro-aggregate. The release of NO toward the blood vessel lumen also inhibits platelet adhesion at the endothelium-blood interface and (in synergy with prostacyclin), exerts a major feedback on platelet aggregation, thereby eliminating the imminent danger of vascular occlusion. In addition, the endothelial barrier prevents the platelet-derived vasoconstrictor substances (thromboxane A2 and serotonin) from reaching the smooth muscle. If the endothelial barrier has been removed, for example as a result of trauma, there is a breakdown in the feedback control of platelet aggregation by NO (and prostacyclin). Aggregation proceeds with the continuous release of serotonin and thromboxane A2, both of which, in the absence of the endothelial barrier, have unrestricted access to smooth muscle. Hence, the smooth muscle contracts and the blood vessel closes down to constitute the vascular phase of haemostasis (Fig. 4).

**Prostacyclin**

Prostacyclin is formed following the activation of phospholipase A2, cyclooxygenase and prostacyclin synthase, primarily in endothelial cells but also in the media and adventitia in response to shear stress, hypoxia, and several mediators that also release NO. Its release depends mainly upon $Ca^{2+}$ release from intracellular stores (Fig. 1). In cultured human endothelial cells, shear stress induces the release of prostacyclin, which is mediated by a pertussis toxin-sensitive $G$ protein. Prostacyclin causes relaxation of vascular smooth muscle by activating adenylate cyclase and increasing the production of cAMP. In most blood vessels, the contribution of prostacyclin to endothelium-dependent relaxation is negligible, and its effect is essentially additive to...
Figure 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) cause the release of 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 toward 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 serotonin, limiting the amount of the monoamine that can diffuse toward the smooth muscle. Finally, the endothelium acts as a physical barrier that prevents the 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 (e.g. 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 haemostasis. +, activation; - , inhibition. (Reproduced with permission.)

that of NO. The two substances act synergistically to inhibit platelet aggregation (Fig. 4).[26-46]

Endothelium-derived hyperpolarizing factor

Electrophysiological studies in various arteries, including the human coronary artery, demonstrate that acetylcholine and other endothelium-dependent dilators cause endothelium-dependent hyperpolarizations and relaxations that are due to a diffusible EDHF, different from NO and prostacyclin[47,48]. The chemical identity of EDHF is not known and several EDHFs may exist. In some blood vessels, epoxyeicosatrienoic acids (EETs) formed from arachidonic acid by the action of cytochrome P-450, may correspond to EDHF[48-51]. The hyperpolarization of smooth muscle cells induced by EDHF is mediated by an increased movement of potassium ions. The type of K⁺ channel involved has not been definitively established, but these channels are more likely to be calcium-dependent rather than ATP-dependent K⁺ channels[48,52,53]. As for NO, the release of EDHF requires an increase in the intracellular Ca²⁺ concentration of the endothelial cells (Fig. 1).

The contribution of hyperpolarization in endothelium-dependent vascular relaxation varies according to the size of the arteries and is prominent in resistance vessels[59,60]. In large arteries, both mediators can contribute to endothelium-dependent relaxations, but the role of NO predominates under normal circumstances. In these arteries, if the synthesis of NO is
Endothelial dysfunction in atherosclerosis

As in several other diseases, the endothelial cells become 
dysfunctional in atherosclerosis[44-47]. This dysfunction 
expresses itself as an impairment in endothelium-
dependent relaxations either due to a reduced release, or 
action, of EDRFs and/or a greater propensity to evoke 
endothelial-dependent contractions.

Regenerated endothelium

In human resistance vessels, among variables including 
age, lipid levels, and blood pressure, age remains 
the most significant predictor of impairment of 
endothelium-dependent vasodilatations[60]. The normal 
aging process induces a turnover and regeneration of 
endothelial cells resulting in an abnormal function. The 
normal life span of an adult human endothelial cell is 
estimated to be about 30 years. After this time cells are 
replaced by regenerated endothelium. These regenerated 
cells have lost some of their ability to release EDRF, 
in particular the ability to respond to platelet aggrega-
tion and thrombin. This conclusion is based on 
in vivo animal studies in which regeneration and the 
characteristics of endothelium-dependent responses were 
monitored following the removal of the coronary 
endothelium[33,67,68]. The regenerated endothelium was 
no longer able to prevent aggregating platelet-induced 
contraction, and responded poorly to serotonin and 
other substances that use the pertussis toxin-sensitive 
pathway controlling the release of EDRF (Fig. 3). In 
cultured regenerated endothelial cells, G, proteins 
are expressed normally, but they have a reduced 
activity[34,69]. The loss of the pertussis toxin-sensitive 
response is selective and does not apply to endothelium-
dependent responses induced by ADP or bradykinin. 
The area of regenerated endothelium becomes a prefer-
ential site for triggering exaggerated vasoconstriction in 
response to serotonin or ergonovine[70].

Mechanism(s)

No impairment of the expression of G, protein a sub-
units was observed in regenerated endothelial cells of 
porcine coronary artery, in endothelial cells with 
impaired release of NO[66,71]. Therefore, the impaired 
release of NO after activation of the pertussis toxins-
sensitive pathway in regenerated endothelial cells, could 
be explained by an altered function of the G, protein[34].

Hypercholesterolaemia and atherosclerosis

Animal data

In experimental animals, hypercholesterolaemia 
induced by high-fat and/or high-cholesterol diets 
impairs endothelium-dependent relaxations in the
rabbitt{8,10,72,73}. By contrast, endothelium-independent relaxations to nitroglycerin, sodium nitroprusside, or adenosine are normal or only slightly impaired. A progressive deterioration of endothelium-dependent relaxations is also observed in genetically hyperlipidaemic rabbits{74}. Endothelium-dependent relaxations are reduced also in the coronary microcirculation of hypercholesterolaemic animals and primates{75,76}. In rabbits, using in vivo ultrasound assessment, impairment of endothelium-dependent vasodilating responses precedes the appearance of echographic atherosclerotic findings{77}.

Clinical data

Endothelium-dependent relaxations are also impaired in humans with atherosclerosis and/or hypercholesterolaemia{78-80}. In isolated coronary arteries from heart transplant recipients, endothelium-dependent relaxations are reduced in atherosclerotic segments in comparison with segments without atherosclerosis{79-91}. Coronarographic studies show that coronary arteries with atherosclerotic lesions constrict to injections of acetylcholine, whereas acetylcholine produces either vasodilation or no change in the coronary diameter in normal patients{92}. Studies with inhibitors of the l-arginine-NO pathway suggest that acetylcholine-induced coronary dilatation in humans is mediated mainly by NO{93-95}. These arteries are capable of dilating in response to nitroglycerin. The paradoxical constriction of atherosclerotic arteries to acetylcholine suggests the occurrence of endothelial dysfunction with a loss of the dilator effect of NO to offset the direct constrictor action of acetylcholine on vascular smooth muscle.

The endothelial dysfunction occurs in the absence of intimal thickening and may be an early and pathogenic event in atherosclerosis{96}. Abnormal coronary responses to acetylcholine are linked directly to the risk factors for coronary artery disease, and the degree of vasoconstriction (or vasodilatation) to acetylcholine varies linearly with the level of plasma cholesterol{86,97}. Endothelial dysfunction occurs predominantly at coronary branching points, which may explain the propensity of these sites of disturbed flow to develop atherosclerosis{98}. Elevated lipoprotein(a) is associated with the impairment of endothelium-dependent vasodilation even when atherosclerotic lesions are not recognizable by angiography{99}. In patients treated with lipid-lowering agents, the improvement in coronary vasodilator response to acetylcholine is related to the degree of protection of low density lipoproteins (LDLs) from oxidation{100}. In peripheral blood vessels, hypercholesterolaemia and other risk factors for atherosclerosis are associated with endothelial dysfunction, as illustrated by abnormal responses to endothelium-dependent vasodilators. The latter can be detected before the development of vascular lesions and even during the first decade of life{101,102}. The endothelium is also dysfunctional in microvessels of patients with atherosclerosis, both in the coronary and peripheral circulations{103,104}. Endothelium-dysfunction is generalized to vascular beds other than those with clinically overt atherosclerosis. Ultrasound techniques demonstrated a relation between brachial vasodilator response and coronary vasodilator response{105}.

Mechanism(s)

Because of the predominance of NO in endothelium-dependent relaxations particularly in large arteries, many studies have focussed on potential alterations of the l-arginine-NO pathway to explain the reduced response to acetylcholine and other endothelial agonists in atherosclerosis. Possible interferences include a reduced intracellular availability of l-arginine, alterations in signalling mechanisms, modification of the expression or the activity of NO synthase, or increased destruction of NO{4,106}. A reduced availability of l-arginine seems unlikely, as endothelial cells contain this substrate in concentrations a thousand times greater (millimolar range) than those that are optimal for constitutive NO synthase activity (micromolar range). l-Arginine supplementation can normalize endothelium-dependent relaxation to acetylcholine in the microcirculation and the aorta of rabbits with an enriched cholesterol diet{107,108}. In hypercholesterolaemic patients, l-arginine improves the response to acetylcholine in coronary microvessels but not in large coronary arteries{103}.

In the early stage of the atherosclerotic process, the endothelial dysfunction appears to be limited to the G protein pathway, which leads to NO formation (Fig. 6). Thus, the ability of regenerated endothelial cells, chronically exposed to high cholesterol levels, to ADP-ribosylate pertussis toxin, is reduced{37}. Consequently, in coronary arteries from hypercholesterolaemic pigs, endothelium-dependent relaxations evoked by agents that activate G{i} protein (serotonin, α-2-adrenoceptor agonists, aggregating platelets, thrombin) are depressed, whereas those induced by ADP, bradykinin, or the calcium ionophore A23187 are preserved{32,35-37,68,73,74}. Oxidized LDLs induce a similar selective endothelial dysfunction in vitro for stimuli activating the G{i} protein pathway, whereas at higher concentrations, they also inhibit endothelium-dependent responses evoked by receptor-independent stimuli (A23187){4,106,109}. This selective dysfunction of the pertussis toxin-sensitive release of NO could be related to a direct inhibitory effect of lysophosphatidylcholine on the function of G{i} proteins and/or to an inhibition of oxidized LDLs on the expression of G{i} proteins{110,111}, which is also decreased in endothelial cells from atherosclerotic human coronary artery{112}. This impairment does not concern smaller coronary microvessels, where, even in normal conditions, the level of the expression of G{i} protein is low{113}. In addition, the decrease in the protective action exerted by the endothelium may be enhanced by the increased production of endothelin-1 by oxidized LDLs{114,115}.

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MODULATED ENDOTHELIAL CELL

Serotonin
UK 14 304 | Endothelin
G_5
5-HT
Adenylate
cyclase

Hyper-
cholesterolemia
Modified LDL

Bradykinin
ADP, ATP
G_3

G_protein
Phospholipase C
ROS

NFKB «—- EDRF-NO
Transcriptional activation

Increased monocyte adhesion
Release of monocyte chemotactic protein (MCP-1)
Release of macrophage colony stimulating factor (M-CSF)
Expression of tissue factor
Production of other cytokines

**Figure 6** Postulated signal transduction processes in a dysfunctional endothelial cell. There is impairment in $G_{i2}$ protein-dependent signalling with a subsequent decrease in the release of endothelium-derived relaxing factor—nitric oxide (EDRF-NO). Furthermore, the resulting increase in cAMP activates the transcription factor NFKB, which induces proatherogenic activity in the endothelial cell. ROS, reactive oxygen species; R, membrane bound receptors; a, a-adrenoceptor; 5-HT, serotonin receptor; ET, endothelin receptor; B, bradykinin receptor; P, Purinergic receptor; G, G protein; LDL, low-density lipoprotein. (Modified from Flavahan and Vanhoutte).  

**Conclusion**

The most important mechanism in the reduction in endothelium-dependent responses is a lower release of NO. Furthermore, as the disease progresses and the artery thickens and stiffens, it becomes increasingly difficult for NO to reach smooth muscle that is still able to relax. Endothelial dysfunction is probably a fundamental initial step in the progression of atherosclerosis. This hypothesis argues that ageing and prolonged exposure to shear stress, coupled with risk factors such as hypertension, smoking and stress, accelerate endothelial aging and hence the process of endothelial regeneration. Consequently, increasingly larger and larger sections of the endothelium become unable to resist platelet adhesion and aggregation and respond less well to thrombin formation. The feedback effect of NO, together with prostacyclin, on platelet aggregation decreases steadily, whereas vasoconstrictor factors (serotonin and thromboxane A2) are released in increasingly greater amounts together with growth factors such as platelet-derived growth factor (PDGF), which is probably responsible for initiating the characteristic morphological changes in atherosclerosis.  

**References**

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