Biology of the Endothelium

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Summary: The endothelium releases factors that control vascular relaxation and contraction, thrombogenesis and fibrinolysis, and platelet activation and inhibition. Maintaining the functional integrity of the endothelium, therefore, is critical for the preservation of blood flow and the prevention of thrombosis. This article reviews the primary endothelium-dependent substances that promote either relaxation (e.g., nitric oxide, prostacyclin) or contraction (e.g., endothelin) of blood vessels, including their physiology, mechanism of effect, and role in endothelial dysfunction. Risk factors for cardiovascular disease, such as hypertension, hypercholesterolemia, diabetes, vascular aging, and estrogen deficiency, are discussed in terms of their contributions to endothelial dysfunction, which may be the initial step in atherogenesis.

Key words: atherosclerosis, endothelial dysfunction, endothelin, hypercholesterolemia, hypertension, nitric oxide, risk factors

Introduction

Since the pioneering work of Furchgott and Zawadzki,[1] the endothelium has been recognized as a major regulator of vascular hemostasis. Endothelial cells, as the inner lining of blood vessels, are strategically located between circulating blood and blood cells and the vascular smooth muscle. In a person with a body weight of 70 kg, the endothelium covers an area of approximately 700 m^2 and weighs about 1 to 1.5 kg.[2] Functional integrity of the endothelium is crucial for the maintenance of blood flow and antithrombotic capacity, because the endothelium releases humoral factors that control relaxation and contraction, thrombogenesis and fibrinolysis, and platelet activation and inhibition. Thus, the endothelium contributes to blood pressure control, blood flow, and vessel patency. It is now clear that impaired endothelial function contributes substantially to cardiovascular disorders such as atherosclerosis, hypertension, and heart failure, which lead to hypoperfusion, vascular occlusion, and end-organ damage.

Physiology of the Endothelium

Endothelium-Derived Relaxing Factors

Stimulation of intact endothelial cells by neurotransmitters, hormones, and substances derived from platelets and the coagulation system causes release of a substance that, in turn, induces relaxation of the underlying vascular smooth muscle (Fig. 1).[1, 3] Furthermore, shear forces generated by circulating blood induce endothelium-dependent vasodilation, which is an important adaptive response of the vasculature during exercise. This endothelium-derived relaxing factor, a diffusible substance with a half-life of a few seconds,[1] has been identified as the free radical, nitric oxide (NO). Nitric oxide is formed from L-arginine by oxidation of the guanidine-nitrogen terminal.[4] The NO-synthesizing enzyme exists in several isoforms in endothelial cells, platelets, macrophages, vascular smooth muscle cells, nerves, and the brain.[5] In endothelial cells, gene expression of NO synthase, although constitutively activated, can be upregulated by shear stress and estrogens. The activity of NO synthase can be inhibited by the circulating amino acid, asymmetrical dimethylarginine (ADMA), which accumulates in patients with renal failure.[6] This observation has been further extended to hypercholesterolemia; increased levels of ADMA were seen in hypercholesterolemic rabbits despite normal renal function,[7] and elevated circulating ADMA was subsequently observed in patients with occlusive peripheral atherosclerotic disease.[8] An inducible isoform of NO synthase exists in vascular smooth muscle and macrophages. When activated by cytokines such as endotoxin, interleukin-1β, and tumor necrosis factor α, this calcium-independent enzyme produces
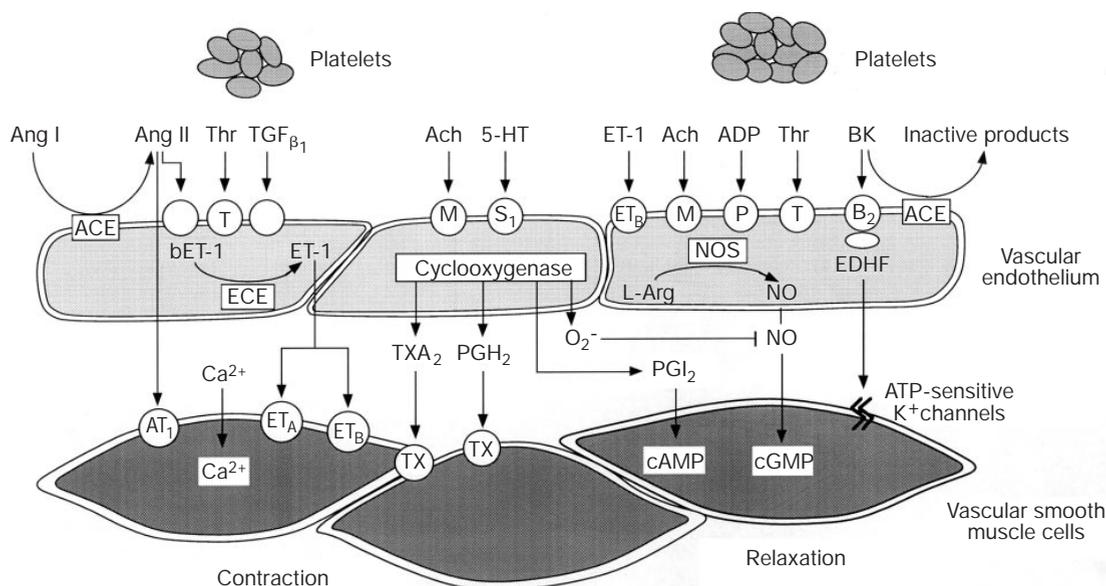
FIG. 1   Vasoactive mediators released by the endothelium. The endothelium produces factors that promote both relaxation (right) and contraction (left). Ang = angiotensin, ACE = angiotensin-converting enzyme, Ach = acetylcholine, ADP = adenosine diphosphate, ATP = adenosine triphosphate, Bk = bradykinin, cAMP/cGMP = cyclic adenosine/guanosine monophosphate, ECE = endothelin-converting enzyme, EDHF = endothelium-derived hyperpolarizing factor, ET = endothelin-1, 5HT = 5-hydroxytryptamine (serotonin), L-Arg = L-arginine, NO = nitric oxide, NOS = nitric oxide synthase, O_2^- = superoxide, PGH_2 = prostaglandin H_2, PGI_2 = prostacyclin, $TGF\beta_1$ = transforming growth factor β_1, Thr = thrombin, TXA_2 = thromboxane A_2. Circles represent receptors (AT = angiotensinergic, B = bradykinergic, ET = endothelin receptor, M = muscarinic, P = purinergic, S = serotonergic, T = thrombin receptor, TX = thromboxane receptor).

large amounts of NO, and hence is activated in inflammatory processes and endotoxic shock.

Endothelium-dependent relaxations due to NO involve formation of cyclic 3',5'-guanosine monophosphate (cGMP) via the soluble enzyme guanylyl cyclase[9] (Fig. 1). Nitric oxide-induced endothelium-dependent relaxation can be pharmacologically inhibited by analogues of L-arginine such as L-N^G-monomethyl arginine (L-NMMA) or L-nitroarginine methyl-ester (L-NAME), which compete with the natural precursor L-arginine at the catalytic site of the enzyme.[5] In isolated arteries, these inhibitors cause endothelium-dependent contractions, whereas in perfused hearts, inhibition of NO formation markedly decreases coronary flow. Local infusion of L-NMMA into the human forearm circulation induces an increase in peripheral vascular resistance. When infused intravenously, L-NMMA induces long-lasting increases in blood pressure. This indicates that the vasculature is in a constant state of vasodilation due to continuous basal release of NO by the endothelium.

In addition to NO, endothelial cells release prostacyclin in response to shear stress, hypoxia, and several substances (see above) that also release NO (Fig. 1). Prostacyclin increases cyclic 3',5'-adenosine monophosphate (cAMP) in smooth muscle and platelets. Its platelet-inhibitory effects play a greater physiologic role than its contribution to endothelium-dependent relaxation. Nitric oxide and prostacyclin synergistically inhibit platelet aggregation, suggesting that the presence of both mediators is required for maximal inhibition of platelet activation.

In the epicardial coronary circulation, inhibitors of the L-arginine pathway do not prevent all endothelium-dependent relaxations, particularly in intramyocardial vessels.[10] Because vascular smooth muscle cells become hyperpolarized during NO-independent relaxations, the existence of endothelium-dependent hyperpolarizing factors has been proposed.[11, 12] However, C-type natriuretic peptide, previously proposed as an endothelium-derived hyperpolarizing factor, does not cause endothelium-dependent hyperpolarization.[13]

Endothelium-Derived Contracting Factors

Soon after endothelium-derived relaxing factor/NO was discovered, it became clear that endothelial cells also can mediate contraction[3] (Fig. 1). Endothelium-derived contracting factors include the 21-amino acid peptide endothelin-1 (ET-1), vasoconstrictor prostanoids such as thromboxane A_2 and prostaglandin H_2, and components of the renin-angiotensin system such as angiotensin II. Three isoforms of the endothelin peptide family exist: endothelin-1, endothelin-2, and endothelin-3. Endothelial cells produce ET-1 exclusively.[14] Translation of messenger RNA generates preproendothelin, which is converted to big endothelin (bET-1) that is further converted by endothelin-converting enzyme (ECE) to the mature peptide ET-1. Four isoforms of this enzyme—ECE-1a, ECE-1b, ECE-1c, and ECE-2—have been cloned.[15, 16] Expression of messenger RNA and release of ET-1 are stimulated by thrombin, transforming growth factor β, interleukin-1, epinephrine, angiotensin II, arginine vaso-

pressin, calcium ionophore, and phorbol ester[14, 17] (Fig. 1).

Endothelin-1 causes vasodilation at lower concentrations but marked and sustained contractions at higher concentrations;[14, 18] in the heart, the latter eventually leads to ischemia, arrhythmias, and death. Intramyocardial vessels are more sensitive to the vasoconstrictor effects of ET-1 than are epicardial coronary arteries, suggesting that endothelin has particular importance in the regulation of flow. Very low circulating levels of ET-1 indicate that most of the peptide is formed locally in the vascular wall. This may be due to the absence of stimuli for endothelin production, the presence of potent inhibitory mechanisms, or the preferential release of endothelin abluminally toward smooth muscle cells.[19] Four inhibitory mechanisms regulating ET-1 production have been delineated: (1) cGMP-dependent inhibition,[17] (2) cAMP-dependent inhibition,[20] (3) an inhibitory factor produced by vascular smooth muscle cells,[21] and (4) inhibition by estrogens via an estrogen-receptor-dependent mechanism.[22] Inhibition of the endothelial L-arginine pathway augments thrombin-induced or angiotensin-induced production of ET-1; conversely, nitrates and atrial natriuretic peptide (which activate particulate guanylyl cyclase) prevent thrombin-induced ET-1 release via a cGMP-dependent mechanism. Endothelin-1 may also promote release of NO and prostacyclin from endothelial cells through ET_B receptors; as a negative feedback mechanism, this process reduces ET-1 production in the endothelium[17] and its vasoconstrictor action in smooth muscle. It is interesting that endothelin inhibits the expression and function of inducible NO synthase.[23]

Two distinct endothelin receptors have been identified: the ET_A- and ET_B-receptors (Fig. 1).[24] Both are G protein-coupled receptors with seven transmembrane domains and are linked to phospholipase C and protein kinase C. Endothelial cells express ET_B-receptors involved in the formation of NO and prostacyclin, which explains the transient vasodilator effects of endothelin when infused into intact organs or organisms. ET_A-receptors and, to some extent, ET_B-receptors mediate contraction and proliferation in vascular smooth muscle. Several endothelin-receptor antagonists have been developed and are currently being clinically evaluated in normal subjects and patients.

The cyclooxygenase pathway also produces endothelium-derived vasoconstrictors. Particularly in veins, but also in the cerebral and ophthalmic circulation, agonists such as arachidonic acid, acetylcholine, histamine, and serotonin can evoke endothelium-dependent contractions that are mediated by thromboxane A_2 or prostaglandin H_2 (Fig. 1).[3] Thromboxane A_2 and prostaglandin H_2 activate the thromboxane receptors in vascular smooth muscle and platelets, thereby counteracting the effects of NO and prostacyclin in both types of cell. In addition, the cyclooxygenase pathway is a source of superoxide anions, which rapidly inactivate NO to form the potent cytotoxic oxidant peroxynitrite.

The endothelium also regulates the activity of the renin-angiotensin system. Angiotensin-converting enzyme (ACE), which converts angiotensin I to angiotensin II, is expressed on the endothelial cell membrane. Angiotensin-converting enzyme is identical to kinase II, which inactivates bradykinin. Angiotensin II can activate endothelial angiotensin receptors; these receptors stimulate the production of ET-1 and other mediators such as plasminogen activator inhibitor.[25] Furthermore, superoxide anion production due to the activation of NADH/NADPH oxidase has recently been linked to angiotensin II-induced hypertension.[26]

Endothelium and Vascular Structure

Removal of endothelial cells by balloon injury invariably leads immediately to deposition of platelets and white blood cells at the site of injury; intimal hyperplasia occurs within days to weeks. This observation suggests that the endothelium regulates vascular structure and that it protects the vessel wall from activation of vascular smooth muscle cells (Fig. 2). Endothelial dysfunction is therefore an important factor in atherosclerosis, restenosis, and hypertensive vascular disease. Vascular structure is determined mainly by vascular smooth muscle cell growth. Endothelial cells may affect vascular structure directly and indirectly. Nitric oxide and prostacyclin inhibit platelet adhesion.[27] Endothelial dysfunction and/or denudation, however, allow platelets to adhere to the vessel wall, where they may cause contraction through the release of thromboxane A_2 and serotonin and may stimulate proliferation and migration of vascular smooth muscle cells via release of platelet-derived growth factor.[28]

Endothelial cells produce growth promoters and growth inhibitors. Under physiologic conditions, the effects of growth inhibitors appear to outweigh those of growth promoters, which may explain why the blood vessel wall is normally quiescent with no proliferation of smooth muscle cells. Heparan sulfates, NO, and transforming growth factor β_1 are potent inhibitors of vascular smooth muscle cell migration and proliferation.[29–31] In contrast, endothelial cells under certain conditions may produce various growth factors, particularly platelet-derived growth factor, epidermal growth factor, and angiotensin II (Fig. 2). These factors may become important in disease states in which the endothelium remains morphologically intact but dysfunctional and may thereby contribute to smooth muscle cell proliferation.

Pathophysiology of the Endothelium

Endothelial Dysfunction: Marker or Mediator?

Endothelial dysfunction is characterized by an imbalance of endothelium-derived relaxing and contracting factors. It may be the cause or consequence of vascular disease and is a hallmark of known cardiovascular risk factors (see below). It is interesting that endothelial dysfunction precedes structural vascular alterations, indicating a protective role of the functionally intact endothelium. While some vessels are particularly prone to developing endothelial dysfunction and atherosclerosis (epicardial coronary arteries, large arteries such as the aorta or iliac artery), others appear to be protected (internal
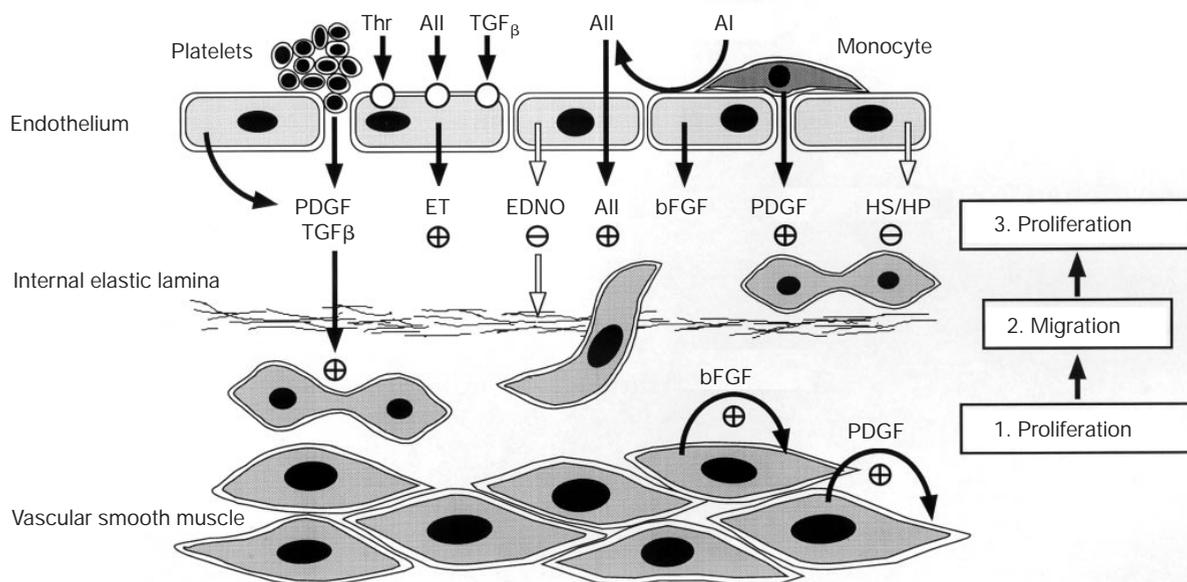
FIG. 2   The endothelium and control of vascular structure. Under normal conditions, the endothelium does not stimulate migration and proliferation of vascular smooth muscle cells. With onset of endothelial dysfunction, platelets and monocytes adhere to the vessel wall, and growth factors are released from these cells as well as from the endothelium. AII = angiotensin II, bFGF = basic fibroblast growth factor, EDNO = endothelium-derived nitric oxide, HP/HS = heparan sulfates, PDGF = platelet-derived growth factor. Other abbreviations as in Figure 1.

mammary artery, brachial artery). This difference may relate to selective alterations due to pulse pressure and/or alterations in endothelial cell function in different areas of the vascular tree. Endothelial cell denudation, however, occurs only in very late stages of atherosclerosis and plaque rupture. These changes in endothelial cell morphology are almost invariably associated with functional alterations and intimal thickening, with accumulation of white blood cells, vascular smooth muscle cells, and fibroblasts and matrix deposition.

Cardiovascular Risk Factors and Endothelial Dysfunction

Hypercholesterolemia: Hypercholesterolemia per se, without atherosclerotic vascular changes, inhibits endothelium-dependent relaxations, which are further reduced in atherosclerosis.[32] It appears that low-density lipoprotein (LDL) is a major determinant of this phenomenon (Fig. 3). Indeed, incubation of isolated coronary arteries with oxidized but not native LDL selectively inhibits endothelium-dependent relaxations to serotonin, aggregating platelets, and thrombin, whereas the response to bradykinin is not affected.[33] A similar diminution of the response can be achieved by pertussis toxin or an inhibitor of NO formation, suggesting defective activation of the L-arginine pathway by G_i protein-coupled receptors.[33, 34] Exogenous L-arginine improves or restores reduced endothelium-dependent relaxation in the presence of oxidized LDL, which suggests that oxidized LDL impairs the activity of NO synthase. The active component of LDL appears to be lysolecithine, which mimics most of the effects of LDL. In vitro experiments in the coronary arteries

of hypercholesterolemic pigs have demonstrated selective dysfunction of endothelium-dependent relaxation in response to serotonin and to aggregating platelets and thrombin. Endothelial dysfunction is more extensive in more advanced stages of atherosclerosis. Experiments in the aorta of hypercholesterolemic rabbits suggest that the overall production of NO is not reduced but rather augmented; however, increased production of NO is inactivated by superoxide radicals produced within the endothelium[35] (Fig. 3). Similar observations have been made in rabbits with fully developed atherosclerosis. Under the conditions of both hypercholesterolemia and atherosclerosis, biologically active NO is markedly reduced, a fact also supported by bioassay experiments with coronary arteries of hypercholesterolemic pigs.[36]

Endothelin is activated in atherosclerotic vascular disease. In hyperlipidemia and atherosclerosis, endothelial cell production of endothelin is increased[37] (Fig. 3), while the expression of endothelin receptors is downregulated.[38] A most likely stimulus for the increased endothelin production is LDL, which increases endothelin gene expression and endothelin release from porcine and human aortic endothelial cells[39] (Fig. 3). Vascular smooth muscle cells, particularly those that migrate into the intima during the atherosclerotic process, also produce endothelin. In cultured vascular smooth muscle cells, endothelin can be released by growth factors such as platelet-derived growth factor and transforming growth factor β_1 and by vasoconstrictors such as arginine vasopressin.[40] Hence, several mediators involved in atherosclerosis stimulate vascular endothelin production, perhaps explaining why plasma endothelin levels are increased and correlate positively with
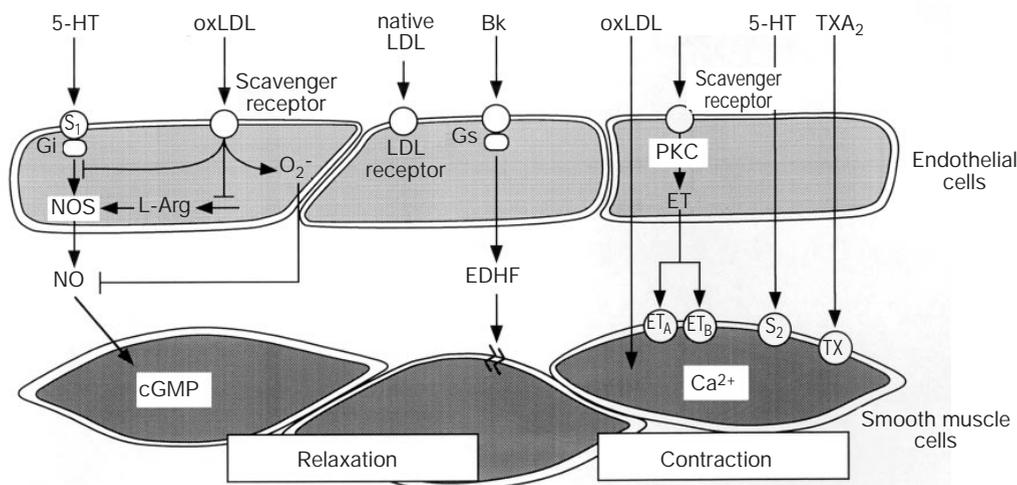
FIG. 3 Endothelial dysfunction in hyperlipidemia and atherosclerosis. The major contributor is oxidized low-density lipoprotein (oxLDL), which, by activating scavenger receptors, impairs the activity of the L-arginine-NO pathway. The mechanism may involve inactivation of G_i proteins (G_i), decreased intracellular availability of L-arginine (L-Arg), and increased breakdown of NO by superoxide (O_2^-). oxLDL further activates endothelin (ET) gene expression and production via protein kinase C (PKC). Other abbreviations as in Figure 1.

the extent of atherosclerotic lesion formation.[37] Furthermore, unstable lesions removed from coronary arteries by atherectomy exhibit marked staining for ET-1.[41] Thus, local vascular endothelin may contribute to both abnormal coronary vasomotion in patients with unstable angina, which may be stimulated by ischemia or thrombin, and to vasoconstriction and the proliferation of vascular smooth muscle cells observed in atherosclerosis.

Hypertension: Endothelial dysfunction in hypertension may contribute to an increase in peripheral vascular resistance (in small arteries) or to vascular complications of the disease (in large and medium-sized conduit arteries). In most models of hypertension, high blood pressure is associated with reduced endothelium-dependent relaxation.[3] Endothelial dysfunction is more prominent in some blood vessels than in others and appears to occur as blood pressure rises; thus, endothelial dysfunction is a consequence rather than a cause of hypertension. In hypertensive subjects, acetylcholine causes paradoxical vasoconstriction of epicardial coronary arteries. The mechanism of endothelial dysfunction differs in various models of hypertension. In the spontaneously hypertensive rat model of genetic hypertension, the activity of the enzyme NO synthase is markedly increased but inefficacious, probably due to increased inactivation of NO by superoxide anion[42] (Fig. 4). In addition, the endothelium of spontaneously hypertensive rats and ren-2 transgenic rats produces increased amounts of prostaglandin H_2, which offsets the effects of NO in vascular smooth muscle and platelets. Whether or not this occurs in humans is uncertain; however, in the forearm circulation of patients with essential hypertension, infusion of a cyclooxygenase inhibitor such as indomethacin enhances vasodilation to acetylcholine.[43] In contrast, salt-induced hypertension is associated with a marked impairment of endothelial NO synthase activity[44]

(Fig. 4). Plasma levels of endothelin remain normal in most patients with hypertension except in the presence of renal failure or atherosclerosis. Increased local vascular production of endothelin, however, is likely; because most of the peptide is released abluminally,[19] plasma levels of endothelin do not necessarily reflect local tissue levels. Vascular endothelin production is reduced in the spontaneously hypertensive rat but increased in angiotensin II-induced hypertension in Wistar-Kyoto rats. In the latter model, functional ECE activity is also increased.[45] However, endothelin by itself does not appear to cause hypertension.[46]

Vascular aging: Aging is a physiologic process associated with an increase in cardiovascular morbidity and mortality even in the absence of known cardiovascular risk factors. This may be related to cellular changes in response to increased oxidative stress[47] or to other factors such as impaired release of vasoactive mediators. In most studies, endothelium-dependent relaxations decrease with aging. In humans, the increase in coronary flow induced by acetylcholine infusion lessens with age.[48] Recent studies have demonstrated that the decline in endothelium-dependent relaxation may be related to a decrease in basal[49] and stimulated[50] release of NO and to reduced expression of the endothelial NO synthase gene. Vascular function is preserved with aging, however, in some arteries such as the femoral artery (Fig. 5).[49] Although plasma levels of endothelin increase with age, the response to endothelin decreases, presumably due to downregulation of receptors in most vessels. Similarly, aging heterogeneously affects functional ECE activity, which may increase in some but not all arteries.[49]

Diabetes: Elevated glucose levels in patients with diabetes cause endothelial dysfunction. The underlying mechanism may involve increased synthesis of endothelin[51] and/or impairment of the L-arginine-NO pathway. Recent studies have
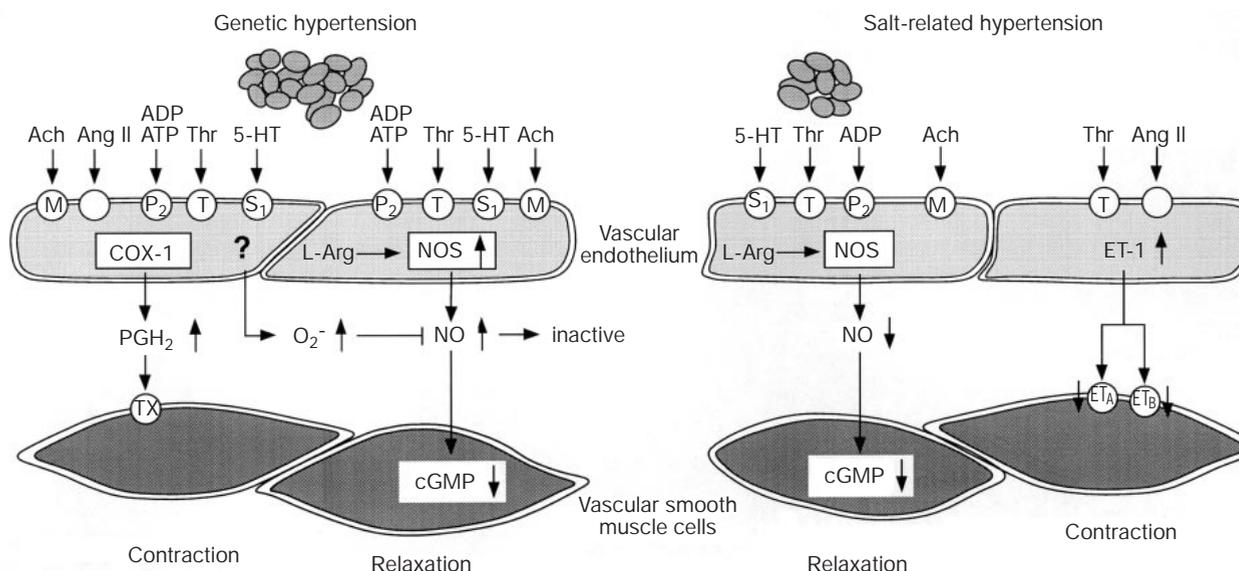
FIG. 4 Endothelial function and hypertension. In spontaneously hypertensive rats (SHR; left), nitric oxide synthase (NOS) activity is increased, but the biological activity of nitric oxide (NO) is reduced, possibly due to inactivation by superoxide (O_2^-). In addition, the production of thromboxane A_2 (TXA$_2$) and prostaglandin H_2 (PGH$_2$) via cyclooxygenase (COX-1) is increased. In contrast, in salt-related hypertension (Dahl rats, Sabra rats, Doca® salt hypertension), NO production is reduced. Production of endothelin (ET-1) is increased in Dahl or Doca® salt hypertension but reduced in SHR. Doca® = desoxycorticosterone acetate. Other abbreviations as in Figure 1.

shown that elevated glucose concentrations increase expression of NO synthase and production of superoxide anion in vitro.[52] Vascular dysfunction due to high glucose levels appears to be mediated in vivo by vascular endothelial growth factor via an NO synthase-linked pathway.[53]

Estrogen deficiency: Estrogen is an important modulator of vascular function. Estrogen replacement therapy is associated with a decreased risk of cardiovascular morbidity and mortality in postmenopausal women.[54] Accordingly, male gender is considered an independent risk factor for coronary artery disease. Estrogen modulates NO synthase activity and the formation of NO in vitro and in vivo. Estrogen deficiency is associated with endothelial dysfunction[55] and increased circulating levels of endothelin.[56] Endothelin can be inhibited by estrogen in vitro[22] and in vivo.[56]

Clinical Implications

Experimental and clinical evidence suggests that endothelial dysfunction is a major determinant for the development and progression of cardiovascular and renovascular diseases. A major goal of therapy in patients with these diseases should be to improve or preserve endothelial function. Furthermore, since endothelial dysfunction occurs prior to structural vascular changes, therapy should be initiated early in patients at risk (e.g., familial hypercholesterolemia, hypertension). Prevention or correction of endothelial dysfunction in cardiovascular disease with agents targeting the endothelium, such as ACE inhibitors, HMG-CoA reductase inhibitors, and estrogen, is likely to improve the clinical outcome in these patients.
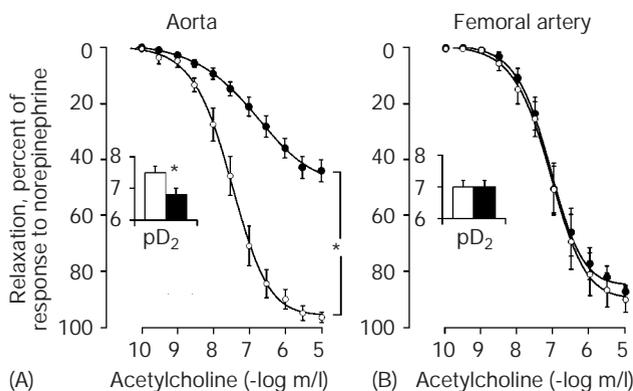

FIG. 5. Endothelial function and vascular aging. Aging impairs NO-mediated endothelium-dependent relaxations to acetylcholine in the aorta of Wistar rats (A), whereas endothelial function in the femoral artery is maintained (B). The different responses indicate an anatomical heterogeneity in the aging process of the endothelium. • Old (n = 6), ○ young (n = 8). Reprinted with permission from Ref. No. 49.

References

1. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;299:373–376

2. Cryer A: Scale and diversity of interaction at the vascular endothelium. In: *Biochemical Interactions of the Endothelium*, p. 1–3. Amsterdam: Elsevier, 1983

3. Lüscher TF, Vanhoutte PM: The *Endothelium: Modulator of*

Cardiovascular Function. Boca Raton, Fla.: CRC Press, 1990

4. Palmer RM, Ashton DS, Moncada S: Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988;333:664–666

5. Nathan C, Xie Q-W: Nitric oxide synthases: Roles, tolls, and controls. *Cell* 1994;78:915–918

6. Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992;339:572–575

7. Bode-Böger SM, Böger RH, Klenke S, Junker W, Frölich JC: Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. *Biochem Biophys Res Commun* 1996;219: 598–603

8. Böger RH, Bode-Böger SM, Thiele W, Junker WKA, Frölich JC: Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. *Circulation* 1997 (in press)

9. Rapoport RM, Draznin MB, Murad F: Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* 1983;306:174–176

10. Tschudi M, Richard V, Bühler FR, Lüscher TF: Importance of endothelium-derived nitric oxide in porcine coronary resistance arteries. *Am J Physiol* 1991;260:H13-H20

11. Vanhoutte PM: Vascular physiology: The end of the quest? *Nature* 1987;327:459–460

12. Campbell WB, Gebremedhin D, Pratt PF, Harder DR: Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 1996;78:415–423

13. Barton M, d'Uscio LV, Bény J-L, Wyss T, Noll G, Lüscher TF: C-type natriuretic peptide hyperpolarizes coronary artery smooth muscle cells by an endothelium-independent mechanism (abstr). *J Cardiol Pharmacol* 1997 (in press)

14. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411–415

15. Ohnaka K, Takayanagi R, Nishikawa M, Haji M, Nawata H: Purification and characterization of a phosphoramidon-sensitive endothelin-converting enzyme in porcine aortic endothelium. *J Biol Chem* 1993;268:26759–26766

16. Xu D, Emoto N, Giaid A, Slaughter C, Kaw S, de Wit D, Yanagisawa M: ECE-1: A membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. *Cell* 1994; 78:473–485

17. Boulanger C, Lüscher TF: Release of endothelin from the porcine aorta: Inhibition of endothelium-derived nitric oxide. *J Clin Invest* 1990;85:587–590

18. Kiowski W, Lüscher TF, Linder L, Bühler FR: Endothelin-1-induced vasoconstriction in humans: Reversal by calcium channel blockade but not by nitrovasodilators or endothelium-derived relaxing factor. *Circulation* 1991;83:469–475

19. Wagner OF, Christ G, Wojta J, Vierhapper H, Parzer S, Nowotny PJ, Schneider B, Waldhaus W, Binder BR: Polar secretion of endothelin-1 by cultured endothelial cells. *J Biol Chem* 1992;267: 16066–16068

20. Yokokawa K, Kohno M, Yasunari K, Murakawa K, Takeda T: Endothelin-3 regulates endothelin-1 production in cultured human endothelial cells. *Hypertension* 1991;18:304–315

21. Stewart DJ, Langleben D, Cernacek P, Cianfione K: Endothelin release is inhibited by coculture of endothelial cells with cells of vascular media. *Am J Physiol* 1990;259:H1928–H1932

22. Barton M, Shaw S, Lüscher TF: 17β-oestradiol inhibits endothelin-1 production stimulated by thrombin but not by angiotensin II in human endothelial cells (abstr). *Hypertension* 1996;28:708

23. Ikeda U, Yamamoto K, Maeda Y, Shimpo M, Kanbe T, Shimada K: Endothelin-1 inhibits nitric oxide synthesis in vascular smooth muscle cells. *Hypertension* 1997;29:65–69

24. Lüscher TF, Oemar BS, Boulanger CM, Hahn AW: Molecular and cellular biology of endothelin and its receptors, part 1. *J Hypertens* 1993;11:7–11

25. Vaughan D, Lazos S, Tong K: Angiotensin regulates the expression of plasminogen activator inhibitor in cultured endothelial cells. *J Clin Invest* 1995;95:995–1000

26. Rajapopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG: Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: Contribution to alterations of vasomotor tone. *J Clin Invest* 1996;97:1916–1923

27. Radomski MW, Palmer RM, Moncada S: The anti-aggregating properties of vascular endothelium: Interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987;92:639–646

28. Ross R: The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* 1993;362:801–809

29. Garg UC, Hassid A: Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 1989;83:1774–1777

30. Battegay EJ, Raines EW, Seifert RA, Bowen-Pope DF, Ross R: TGF-beta induces bimodal proliferation of connective tissue cells via complex control of an autocrine PDGF loop. *Cell* 1990;63: 515–524

31. Castellot JJ Jr, Beefer DL, Rosenberg RD, Karnovsky MJ: Structural determinants of the capacity of heparin to inhibit the proliferation of vascular smooth muscle cells. *J Cell Physiol* 1984;120: 315–320

32. Harrison DG, Freiman PC, Armstrong ML, Marcus ML, Heistad DD: Alterations of vascular reactivity in atherosclerosis. *Circ Res* 1987;61:1174–1180

33. Tanner FC, Noll G, Boulanger CM, Lüscher TF: Oxidized low density lipoproteins inhibit relaxations of porcine coronary arteries: Role of scavenger receptor and endothelium-derived nitric oxide. *Circulation* 1991;83:2012–2020

34. Shimokawa H, Flavahan NA, Vanhoutte PM: Natural course of the impairment of endothelium-dependent relaxations after balloon endothelium removal in porcine coronary arteries: Possible dysfunction of a pertussis toxin-sensitive G protein. *Circ Res* 1989; 65:740–753

35. Minor R Jr, Myers PR, Guerra R Jr, Batas JN, Harrison DG: Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *J Clin Invest* 1990;86:2109–2116

36. Shimokawa H, Vanhoutte PM: Hypercholesterolemia causes generalized impairment of endothelium-dependent relaxation to aggregating platelets in porcine arteries. *J Am Coll Cardiol* 1989;13: 1402–1408

37. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JCJ: Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med* 1991;325:997–1001

38. Winkles JA, Alberts GF, Brog E, Libby P: Endothelin-1 and endothelin receptor mRNA expression in normal and atherosclerotic human arteries. *Biochem Biophys Res Commun* 1993;191:1081–1088

39. Boulanger CM, Tanner FC, Bea ML, Hahn AW, Werner A, Lüscher TF: Oxidized low density lipoproteins induce mRNA expression and release of endothelin from human and porcine endothelium. *Circ Res* 1992;70:1191–1197

40. Hahn AW, Resink TJ, Scott-Burden T, Powell J, Dohi Y, Bühler FR: Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: A novel autocrine function. *Cell Regul* 1990; 1:649–659

41. Zeiher AM, Ihling C, Pistorius K, Schächinger V, Schaefer H-E: Increased tissue endothelin immunoreactivity in atherosclerotic lesions associated with acute coronary syndromes. *Lancet* 1994; 344:1405–1406

42. Nava E, Noll G, Lüscher TF: Increased cardiac activity of nitric oxide synthase in spontaneous hypertension. *Circulation* 1995;91: 179–181

43. Taddei S, Virdis A, Mattei P, Salvetti A: Vasodilation to acetyl-

choline in primary and secondary forms of human hypertension. *Hypertension* 1993;21:929–933

44. Hayakawa H, Raij L: The link among nitric oxide synthase activity, endothelial function, and aortic and ventricular hypertrophy in hypertension. *Hypertension* 1997;29:235–241

45. Barton M, Shaw S, d'Uscio LV, Moreau P, Lüscher TF: Endothelin ETA receptor blockade prevents angiotensin II-induced increases in ECE activity and endothelin-1 levels in rat arteries. *Nephrology* 1997;3:S32

46. Hocher B, Thöne-Reinecke C, Rohmeiss P, Schmager F, Slowinski R, Burst V, Siegmund F, Quertermous T, Bauer C, Neumayer HH, Schleuning WD, Theuring F: Endothelin-1 transgenic mice develop glomerulosclerosis, interstitial fibrosis, and renal cysts but not hypertension. *J Clin Invest* 1997;99:1380–1389

47. Ames BN, Shigenaga MK: Oxidants are a major contributor to aging. *Ann NY Acad Sci* 1992;663:85–96

48. Zeiher AM, Drexler H, Saurbier B, Just H: Endothelium-mediated coronary blood flow modulation in humans: Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. *J Clin Invest* 1993;92:652–662

49. Barton M, Cosentino F, Brandes RP, Moreau P, Shaw S, Lüscher TF: Anatomical heterogeneity of vascular aging: Role of nitric oxide and endothelin. *Hypertension* 1997;30:817–824

50. Tschudi MR, Barton M, Bersinger NA, Moreau P, Noll G, Cosentino F, Malinski T, Lüscher TF: Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. *J Clin Invest* 1996;96:899–905

51. Yamaguchi T, Ohnaka K, Takayanagi R, Umeda F, Nawata H: Enhanced secretion of endothelin 1 by elevated glucose levels from cultured bovine endothelial cells. *FEBS Lett* 1990;267: 16–18

52. Cosentino F, Hishikawa K, Katusic ZS, Lüscher TF: High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 1997; 96:25–28

53. Tilton RG, Kawamura T, Chang KC, Ido Y, Bjercke RJ, Stephan CC, Brock TA, Williamson JR: Vascular dysfunction induced by elevated glucose levels in rats is mediated by vascular endothelial growth factor. *J Clin Invest* 1997;99:2192–2202

54. Stampfer MJ, Colditz GA, Willet WC, Manson JE, Rosner B, Speizer FE, Hennekens CH: Postmenopausal estrogen therapy and cardiovascular disease: Ten-year follow-up from the Nurses' Health Study. *N Engl J Med* 1991;325:726–762

55. Taddei S, Virdis A, Ghiadoni L, Mattei P, Sudano I, Bernini G, Pinto S, Salvetti A: Menopause is associated with endothelial dysfunction in women. *Hypertension* 1996;28:576–582

56. Ylikorkala O, Orpana A, Puolakka J, Pyorala T, Viinikka L: Postmenopausal hormonal replacement decreases plasma levels of endothelin-1. *J Clin Endocrinol Metab* 1995;80:3384–3387