THE WIGGERS AWARD LECTURE

Endothelial dysfunction: a multifaceted disorder

Michel Féletou and Paul M. Vanhoutte

Department of Angiology, Institut de Recherches Servier, Suresnes, France and Department of Pharmacology, Faculty of Medicine, University of Hong Kong, Hong Kong, China

Féletou, Michel, and Paul M. Vanhoutte. Endothelial dysfunction: a multifaceted disorder. Am J Physiol Heart Circ Physiol 291: H985–H1002, 2006.—Endothelial cells synthesize and release various factors that regulate angiogenesis, inflammatory responses, hemostasis, as well as vascular tone and permeability. Endothelial dysfunction has been associated with a number of pathophysiological processes. Oxidative stress appears to be a common denominator underlying endothelial dysfunction in cardiovascular diseases. However, depending on the pathology, the vascular bed studied, the stimulant, and additional factors such as age, sex, salt intake, cholesterolemia, glycemia, and hyperhomocysteinemia, the mechanisms underlying the endothelial dysfunction can be markedly different. A reduced bioavailability of nitric oxide (NO), an alteration in the production of prostanooids, including prostacyclin, thromboxane A2, and/or isoprostanes, an impairment of endothelium-dependent hyperpolarization, as well as an increased release of endothelin-1, can individually or in association contribute to endothelial dysfunction. Therapeutic interventions do not necessarily restore a proper endothelial function and, when they do, may improve only part of these variables.

nitric oxide; prostaglandins; endothelium-derived hyperpolarizing factor; endothelium-derived contracting factor; endothelin; oxidative stress; superoxide anion; hypertension; regenerared endothelium; angioplasty

Although the endothelium forms a single layer of cells, the total volume of the endothelial cells of the human body is comparable to that of the liver (100). In response to various substances (released by autonomic and sensory nerves or platelets), circulating hormones, autacoids, cytokines, and drugs, as well as to physical and chemical stimuli (e.g., changes in pressure, shear stress, and pH), endothelial cells synthesize and release various factors that modulate angiogenesis, inflammatory responses, hemostasis, as well as vascular tone and permeability. The vasoactive factors include relaxing [adenosine, prostacyclin (PGI2), nitric oxide (NO), hydrogen peroxide (H2O2), epoxyeicosatrienoic acids (EETs), and C-natriuretic peptide (CNP)] and contracting [e.g., thromboxane A2, isoprostanes, 20-hydroxyeicosatetraenoic acid, superoxide anion (O2−), H2O2, endothelin-1, angiotensin II, and uridine adenosine tetraphosphate] factors. Endothelial cells also directly communicate with smooth muscle cells via myoendothelial gap junctions that allow not only the spread of electrotonic tone [such as endothelium-dependent hyperpolarizations, i.e., endothelium-derived hyperpolarizing factor (EDHF)-mediated responses] but also the transfer of ions or small molecules such as calcium and cyclic nucleotides (56).

As a major regulator of local vascular homeostasis, the endothelium maintains the balance between vasodilatation and vasoconstriction, inhibition and promotion of the proliferation and migration of smooth muscle cells, prevention and stimulation of the adhesion and aggregation of platelets, as well as thrombogenesis and fibrinolysis (40). Upsetting this tightly regulated balance leads to endothelial dysfunction.1

ENDOTHELIAL DYSFUNCTION

The term “endothelial dysfunction” was coined in the mid-eighties, following the major breakthrough by Furchgott and Zawadzki (66) who discovered that acetylcholine requires the presence of the endothelial cells to relax the underlying vascular smooth muscle. Before the factor released by acetylcholine [first termed as endothelium-derived relaxing factor (EDRF)] was identified as nitric oxide (NO), it had already been observed that the endothelium-dependent relaxations in the aorta of hypertensive rats (147, 289) and hypercholesterolemic rabbits (101, 276) were impaired. Similar observations were made in human coronary arteries of atherosclerotic patients (149), and it was suggested that this endothelial dysfunction could be an early marker of atherosclerosis (108). Since then the term “endothelial dysfunction” has been referred to in

1 The Carl J. Wiggers Award is in honor of the Cardiovascular Section’s founder, Carl J. Wiggers, whose research defined fundamental pressure-flow relationships in the cardiovascular system. The award is presented to a scientist who is a Fellow of the Cardiovascular Section of the APS, who has made outstanding and lasting contributions to cardiovascular research throughout his/her career. The 2006 Carl J. Wiggers Award was presented to Dr. Paul Vanhoutte.
the scientific literature more than 20,000 times (PubMed search, November 2005) and has been associated not only with hypertension or atherosclerosis, but also with physiological and pathophysiological processes, including aging, heart and renal failure, coronary syndrome, microalbuninuria, dialysis, thrombosis, intravascular coagulation, preeclampsia, Type I and Type II diabetes, impaired glucose tolerance, insulin resistance, hyperglycemia, obesity, postprandial lipemia, hypercholesterolemia, hyperhomocysteinemia, elevated asymmetric dimethylarginine plasma levels, inflammation, vasculitis, infections, sepsis, rheumatoid arthritis, periodontitis, trauma, transplantation, low birth weight, postmenopause in women, mental stress, sleep apnea syndrome, smoking, nitrate tolerance, glucocorticoids, and so on.

In humans, endothelial dysfunction can be assessed biochemically by dosing different markers (e.g., adhesion molecules, cytokines, and prostanoids) in the blood or functionally by measuring endothelium-dependent dilatation in vitro (isolated arteries) and in vivo either in response to agonists or to changes in flow in the forearm, coronary, or peripheral circulation. The measurement of flow-mediated dilatation, although an indirect method to evaluate endothelial function, is noninvasive and therefore has been extensively used (41, 53). Endothelial dysfunction, assessed directly in coronary arteries or by flow-mediated dilatation in the brachial artery, predicts long-term cardiovascular events in patients with coronary disease, hypertension, heart failure, or atherosclerosis (31, 57, 73, 88, 188, 210, 232). However, whether or not endothelial dysfunction is a true independent predictor, a risk factor, a risk marker, or a surrogate end point is still a matter of debate (52, 55, 155).

MECHANISMS UNDERLYING ENDOTHELIAL DYSFUNCTION

Although endothelial dysfunction occurs in many different disease processes, oxidative stress can be identified as a common denominator (77, 78). Reactive oxygen species play a central role in vascular physiology and pathophysiology. NO, O$_2^-$, the hydroxyl radical (·OH), H$_2$O$_2$, and peroxynitrite (ONOO$^-$) are produced in the vasculature under both normal and stress conditions such as inflammation or injury. O$_2^-$ can be generated by different enzymes (e.g., NADPH oxidase, xanthine oxidase, cyclooxygenases, NO synthases, cytochrome P450 monooxygenases, and enzymes of the mitochondrial respiratory chain) in virtually all cell types, including vascular smooth muscle and endothelial cells. Superoxide either spontaneously or enzymatically [through dismutation by superoxide dismutase (SOD)] is reduced to the uncharged H$_2$O$_2$. H$_2$O$_2$ in the presence of the enzyme catalase or glutathione peroxidase is then dismutated into water and oxygen. In the presence of transition metals (copper, iron) or superoxide anions, H$_2$O$_2$ generates, through the Fenton or Haber-Weiss reaction, respectively, the highly reactive hydroxyl radicals that can be scavenged by mannitol or dimethylthiourea. Hydroxyl radicals cause cell damage through the peroxidation of lipids and sulfhydryl groups. When NO and O$_2^-$ are produced in close vicinity, they interact to form ONOO$^-$, a potent oxidant also capable of oxidizing sulfhydryl groups as well as nitrating and hydroxylating aromatic groups, including tyrosine, tryptophan, and guanine (305; Figs. 1 and 2).

Reactive Oxygen Species and Endothelium-Derived Vasodilators

Reactive oxygen species regulate several classes of genes, including those controlling the formation of adhesion molecules, chemotactic substances, and antioxidant enzymes. Furthermore, as they activate metalloproteinases and tilt the endothelial balance toward vasoconstriction, their (over)production is of particular relevance to vascular pathologies (77). Reactive oxygen species can inhibit the three major endothelium-dependent vasodilator pathways, i.e., NO, prostacyclin, and EDHF. Superoxide anions not only reduce the bioavailability of NO but also directly inhibit its main target, soluble guanylyl cyclase, inactivates the prostacyclin synthase by tyrosine nitration, and further enhances oxidative stress by inhibiting superoxide dismutases (169, 305). The activity of calcium-activated potassium channels involved in EDHF-mediated responses is decreased by the chronic action of superoxide anion (130), and oxidant stress decreases the electrotonic signaling by means of myoendothelial and smooth muscle gap junctions by interacting with connexins 37, 40, and 43 (79). Likewise, peroxynitrite decreases the EDHF component of flow-mediated vasodilatation in mice coronary arteries (146).

Additional Effects of Reactive Oxygen Species

In addition, reactive oxygen species promote the contraction of vascular smooth muscle cells by facilitating the mobilization
of calcium and increasing the sensibility of the contractile proteins to calcium ions (109, 235). Superoxide anion mediates stretch and agonist-induced endothelium-dependent contractions in canine cerebral arteries (115). They can also activate endothelial enzymes, including cyclooxygenases, which produce endothelium-derived contracting factors [EDCF (270)]. Superoxide anion-dependent oxidative modification of polyunsaturated fatty acids produces isoprostanes, members of a family of prostaglandin isomers (164). Isoprostanes are not only markers of oxidative stress (195) but are also bioactive molecules that produce vasoconstriction by activating thromboxane-prostanoid (TP) receptors on vascular smooth muscle (296). H$_2$O$_2$, which is produced by both the endothelium and the smooth muscle cells, is not only a relaxing and a hyperpolarizing factor (225), but depending on the species, the artery, and concentration, it can also cause depolarization of the smooth muscle and vasoconstriction (56).

However, although oxidative stress is a common denominator of endothelial dysfunction, depending on the pathology involved, the mechanism(s) underlying the impairment of endothelium-dependent responses can be markedly different. To exemplify this phenomenon, the present brief review will focus on hypertension and balloon catheter angioplasty leading to endothelial cell regeneration.

**HYPERTENSION**

*Spontaneous Hypertensive and Stroke-Prone Hypertensive Rats*

**Endothelin.** Endothelin-1 is one of the most potent known vasoconstrictor peptides produced and released mainly by endothelial cells. This peptide is also involved in proliferation and hypertrophy of vascular smooth muscle cells (211, 291). In the spontaneously hypertensive rat (SHR), plasma endothelin levels are similar or slightly elevated compared with those of normotensive Wistar-Kyoto (WKY; 1, 13, 106, 234). By contrast, in the stroke-prone spontaneously hypertensive rat (SHRSP), a more severe model of genetic hypertension, an increase in the circulating level of the peptide is observed consistently (1, 211). In this latter strain, endothelin contributes to end-organ damage, vascular remodeling but only moderately to the increase in blood pressure (211).

**Endothelium-dependent relaxations. AORTA.** In the aorta of SHR, the endothelium-dependent relaxations are impaired and are associated with the generation of EDCF with no or little alteration in the production of NO. The release of cyclooxygenase-derived contractile prostanoids, but not that of endothelin, is responsible for these endothelium-dependent contractions (153, 270). The mechanisms underlying endothelium-dependent contractions in the SHR aorta involve the production of reactive oxygen species, the activation of endothelial cyclooxygenase-1, the diffusion of EDCF, and the subsequent stimulation of TP receptors on vascular smooth muscle (Fig. 3). Inhibitors of thromboxane synthase do not affect the endothelium-dependent contraction to acetylcholine, indicating that thromboxane A$_2$ is not the EDCF released following muscarinic receptor activation (7, 68, 72, 114, 125, 126, 153, 252, 255, 292, 293, 295, 296). The massive release of prostacyclin, which in the SHR aorta paradoxically is not a relaxing but a contracting prostaglandin (PG), and also that of the endotheroxide PGH$_2$, accounts for the biological activity of EDCF (68, 72, 197; Figs. 3 and 4). Indeed, prostacyclin evokes no or little relaxation in the aorta of aging SHR and WKY most likely because the expression of the PG$_I_2$ receptor (IP receptor) gene decreases with age and is systematically less expressed in the SHR than in the WKY at any given age (175). However, in response to other stimuli, such as endothelin, the production of thromboxane A$_2$ also can contribute to EDCF-mediated responses (237).

In the aorta of young SHRSP, in the developmental stage of hypertension, the endothelium-dependent relaxations and the production of NO in response to cholinergic agonists are enhanced paradoxically, although the so-called “basal release” of NO is already impaired (48, 179, 260). Endothelial dysfunction develops later involving both a decrease in NO bioavailability (with no changes in the expression of NO synthase) and the cyclooxygenase-dependent production of an EDCF associated with an enhanced production of prostacyclin and thromboxane A$_2$ (173, 174, 179, 222). Additionally, the decreased expression of soluble guanylyl cyclase B$_2$-subunit in the smooth muscle may alter the efficacy of endothelin-derived NO to cause relaxation (148, 201).
OTHER ARTERIES. In the mesenteric and renal arteries of the SHR, stimulation with acetylcholine involves again the production of an EDCF with no or little alteration in the production of NO. However, there is a marked attenuation of the EDHF-mediated component (47, 63, 64, 87, 99, 150, 151, 156). This decrease in EDHF-mediated response has been associated with, but not yet causally linked, to a change in the expression profile of gap junctions in endothelial cells.

Fig. 3. Endothelial dysfunction in aorta of SHR rats. A: acetylcholine-induced, endothelium-dependent relaxations in isolated and phenylephrine-contracted aortic rings from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) (solid lines, with endothelium; dashed lines, without endothelium. Modified from Ref. 294). B: endothelium-dependent contractions to acetylcholine in aortic rings of SHR [presence of N\(^\text{G}\)-nitro-L-arginine (L-NNA): 100 \(\mu\text{M}\)]. Thromboxane prostanoid (TP) receptor antagonist S-18886 and the specific COX-1 inhibitor valeryl salicylate but not the specific COX-2 inhibitor NS-398 abolish acetylcholine-induced, endothelium-dependent contractions (modified from Ref. 292). C: prostacyclin release (6-keto-PGF\(_1\alpha\), left y-axis) and acetylcholine-induced contraction (right y-axis) in aortic rings with and without endothelium of SHR. Contractions were obtained in presence of L-NNA (100 \(\mu\text{M}\)) (modified from Ref. 72).

Fig. 4. Endothelium-dependent effects of acetylcholine in rat aorta. Left: endothelium-dependent relaxations in normotensive rats. Right: cyclooxygenase-dependent, endothelium-dependent contractions to acetylcholine in SHR aorta. PGI\(_2\), prostacyclin; R, receptor; IP, PGI\(_2\) receptor; TP, TP receptor; PLA\(_2\), phospholipase A\(_2\); AA, arachidonic acid; COX\(_1\), cyclooxygenase 1; S-18886, antagonist of TP receptors; M, muscarinic receptor; PGIS, prostacyclin synthase; PGH\(_2\), endoperoxides; sGC, soluble guanylyl cyclase; AC, adenyl cyclase; SR, sarcoplasmic reticulum; +, activation; -, inhibition; ?, unknown site of formation.
Fig. 5. Endothelial dysfunction in SHR rats. In SHR, forces exerted by flowing blood and/or receptor stimulation increases endothelial intracellular calcium concentration, which activates many enzymes capable of generating O$_2^-$; they include COX, LOX, cytochrome P450 monoxygenases (P450), and NOS. O$_2^-$ are reduced enzymatically into H$_2$O$_2$ by superoxide dismutases (SOD). In rat peripheral arteries such as the mesenteric artery, endothelium-derived hyperpolarizing factor (EDHF)-mediated responses can be explained by activation of endothelial calcium-activated potassium channels. This produces not only endothelial hyperpolarization conducted through myoendothelial gap junctions to the underlying vascular smooth muscle but also accumulation of potassium ions in the intercellular space. These two mechanisms are not necessarily mutually exclusive and, in a given blood vessel, they can occur simultaneously, sequentially or even synergistically (56). In SHR rats, the reduced EDHF-mediated response, possibly because of a reactive oxygen species (ROS)-dependent decrease in myoendothelial gap junction communication, as well as the production of EDCF, most likely PGH$_2$ and prostacyclin (see Figs. 2 and 3), explain the impairment of the endothelium-dependent relaxations in these peripheral arteries. PLA$_2$, phospholipase A$_2$; AA, arachidonic acid; P450, cytochrome P450 monoxygenases; R, receptor; ACh, acetylcholine; BK, bradykinin; SP, substance P; IP$_3$, inositol trisphosphate; SR, sarcoplasmic reticulum; TRP, transient receptor potential channel; CX, connexin; SK3, small conductance calcium-activated potassium channel formed by SK3 α-subunits; IK1, intermediate conductance calcium-activated potassium channel formed by IK1 α-subunits; Kir2.1, inward rectifying potassium channel constituted of Kir2.1 α-subunits.

The Effects of pharmacological and therapeutic agents. The amplitude of the acetylcholine-induced, endothelium-dependent contraction in the SHR and WKY aorta is positively correlated with the level of arterial blood pressure (105). However, chronic treatment with either a cyclooxygenase inhibitor or a TP receptor antagonist does not prevent the establishment of hypertension and does not decrease arterial blood pressure in SHR rats (141, 230, 297) but restores the endothelium-dependent relaxation in the aorta (255). These results suggest that, in the SHR and the SHRSP, the production of EDCF is genetically determined and exacerbates the endothelial dysfunction associated with chronic hypertension. This interpretation is reinforced by the observations that 1) in SHRSP, hypertension precedes the occurrence of endothelium-dependent contractions (180); and 2) endothelium-dependent contractions appear also in arteries of aging normotensive WKY (65, 72, 89, 126).

In both the SHR and the SHRSP, the production of NO (in the vasculature, kidneys, heart, and central nervous system) is generally not affected and can even be increased, but the enhanced production of superoxide anion reduces its bioavailability (80, 259, 266). Endothelial xanthine oxidase, NAPDH oxidase, as well as endothelial NO synthase (eNOS) itself [when uncoupled because of substrate (L-arginine) deficiency and/or because of oxidation or deficiency of the cofactor, tetrahydrobiopterin (30, 131, 274, 290)] can contribute to the generation of superoxide anions (28, 83, 119, 135, 233, 299). In the SHRSP, this increase in reactive oxygen species production possibly is magnified by a decrease in the expression of glutathione S-transferase μ-type 1 (Gstm1), an enzyme involved in the cellular defense against oxidative stress, because of a single nucleotide polymorphism (R202H) in the promoter region of this gene (161).
Vascular oxidative stress precedes the establishment of high blood pressure (171). Furthermore, in both SHR and SHRSP, scavenging superoxide anion with membrane-permeable SOD mimetics (Tempol, M40403) inhibits apoptosis of the endothelial cells and prevents rarefaction of the microvessels and the installation of hypertension. When given acutely in hypertensive animals, these agents decrease arterial blood pressure and restore endothelium-dependent relaxations (39, 123, 187, 216). In these two strains of genetically hypertensive rats, angiotensin-converting enzyme inhibitors and antagonists of AT1 angiotensin receptors are effective antihypertensive agents (22, 267). Angiotensin II is a potent activator of NADPH oxidase in vascular cells (138). Preventing the generation of reactive oxygen species, for instance by deleting a subunit of the NADPH oxidase \([p47(phox)−/− \text{ mice}]\), induces resistance to angiotensin II-induced hypertension and markedly reduces the associated endothelial generation of superoxide anions (134). In the SHR and SHRSP, both angiotensin-converting enzyme inhibitors and antagonists of AT1 angiotensin receptors decrease the generation of superoxide anions (20, 251, 287). They diminish the amplitude of endothelium-dependent contractions (142, 200) and restore the amplitude of both NO- and EDHF-mediated endothelium-dependent relaxations (36, 75, 177, 200). The improvement in EDHF-mediated responses may involve normalization of the expression of gap junctions in the endothelial cells because the decreased expression of connexins 37 and 40 observed in the SHR compared with the WKY is corrected by candesartan (113; Fig. 5). Qualitatively similar results \[i.e., a decrease in blood pressure, reduced oxidative stress, and improvement of endothelium-dependent relaxations\] have been reported following chronic treatments with vasopetidase inhibitors (25, 102), calcium channel blockers (76, 174, 220, 265), raloxifene, a selective estrogen receptor modulator (283), as well as with tetrahydrobiopterin supplementation (92). Thus these experimental data confirm that in both SHR and SHRSP, oxidative stress, endothelial dysfunction, and hypertension are closely associated.

However, these variables do not always change in unison and can be affected differentially by other pharmacological agents. For instance, chronic treatment with inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase produce no or minimal changes in the arterial blood pressure of the SHR or SHRSP (112, 117, 282). Statins reduce the expression of p22phox, an essential NADPH oxidase subunit, and upregulate the expression of eNOS, decreasing superoxide anion production and enhancing NO bioavailability (282). This restores the NO-dependent component of the endothelium-dependent relaxations but does not affect the impaired EDHF-mediated responses (112, 282). Similarly, in the SHRSP, chronic treatment with an inhibitor of phosphodiesterase type V attenuates endothelial dysfunction by decreasing oxidative stress and increasing the bioavailability and effectiveness of NO, without affecting systolic arterial blood pressure (34). In the SHR, the inhibition of poly(ADP-ribose) polymerase \[PARP, the enzyme activated following DNA-single strand breakage after superoxide and/or peroxynitrite generation\] improves endothelium-dependent and NO-mediated relaxations without affecting the elevated arterial blood pressure (182) and without decreasing oxidative stress (181). The beneficial effects of the inhibition of poly(ADP-ribose) polymerase is attributed to preserved endothelial levels of NAD\(^+\) and consequently those of ATP (278). Conversely, vasodilators (hydralazine or ecarazine), whether or not in association with diuretics, markedly reduce arterial blood pressure and oxidative stress (42) but do not (11, 36, 46) or only partially restore endothelium-dependent relaxations and, if so, by a modest improvement of the EDHF-mediated responses (74, 103, 177).

Other Animal Models of Hypertension

In other animal models of hypertension such as chronic angiotensin II infusion, transgenic animals overexpressing the renin and the angiotensinogen gene, renovascular hypertension (one-kidney, one-clip, Goldblatt hypertension, renal mass reduction) but also in the so-called “low renin hypertension” models (Dahl salt-sensitive hypertension, deoxycorticosterone acetate-salt hypertension, endothelin-1-induced hypertension), the high arterial blood pressure generally also is associated with an increased generation of reactive oxygen species and an impairment of endothelium-dependent relaxations (136). Again, the endothelial dysfunction can involve markedly different mechanisms depending on the animal model of hypertension or the vascular bed studied.

In the hypertensive salt-sensitive Dahl rat, the endothelin (ET) system is activated and ETA receptor antagonists prevent the endothelial dysfunction and part of the increase in arterial blood pressure (8, 50). Oxidative stress, through an increased expression of NADPH oxidase, could be the common denominator of hypertension and endothelial dysfunction because antioxidants prevent the increase in blood pressure caused by the high-salt diet and the associated endothelial dysfunction (180, 301). In the aorta and mesenteric artery, the production of NO is reduced without any evidence for the production of an EDCF (50, 86, 152). The inhibition of NO synthase by the enhanced production of carbon monoxide, due to an increased expression of heme oxygenase-1, may partially explain the decrease in NO synthesis (110). By contrast, an endotelin-derived contractile PG \[most likely PGH\(_2\) and/or thromboxane A\(_2\)\], acting on TP receptors, contributes in part to the impairment of endothelium-dependent relaxations of renal and carotid arteries (9, 303, 304). Additionally, in the afferent arterioles as well as in mesenteric and coronary arteries, EDHF-mediated responses are attenuated compared with control animals or to salt-sensitive animals fed with a low-salt diet (67, 178, 180). However, in nongenetically selected animals, such as the weanling Sprague-Dawley rat, a high-salt diet also induces hypertension but, in contrast to the Dahl salt-sensitive rats, causes no apparent endothelial dysfunction in the mesenteric artery because an increase in the EDHF component compensates for the decrease in the NO-mediated relaxation (228).

In the hypertensive NOS-3 knockout mice (96), plasma levels of endothelin are not affected compared with the wild-type controls (132). The generation of reactive oxygen species is not increased in the endothelial cells of this murine model (129), and there is no evidence for the production of EDCF. In the aorta of this strain, endothelium- and NO-dependent relaxations are absent, whereas in the mesenteric artery both prostacyclin and EDHF-mediated responses compensate the absence of the NO production (19, 33, 45, 94, 95, 219, 281). Such compensatory mechanisms have also been observed in some, but not all, models of hypertension caused by NO synthase.
inhibitors. In the perfused mesentery of rats treated chronically with an NO synthase inhibitor, the decrease in the availability of NO is compensated for by an increased EDHF component (154, 204). By contrast in the guinea pig, NO synthase inhibitors are potent pressor agents (4), but chronic treatment with one of these agents, nitro-L-arginine-methyl ester, does not significantly affect endothelium-dependent hyperpolarizations, at least in the isolated carotid artery (37).

**Human Hypertension**

In the human, reactive oxygen species are elevated in many cardiovascular diseases, including essential, renovascular, and malignant hypertension as well as preeclampsia. An increased production of reactive oxygen species, a decreased antioxidant activity, and a reduced ability to scavenge oxygen-derived free radicals all contribute to oxidative stress (261). The renin-angiotensin system is likely to play a major role in the generation of reactive oxygen species through the activation of NADPH oxidase (136). The involvement of the endothelin system possibly further exacerbates oxidative stress (193). In the human, endothelin plasma levels are not correlated with blood pressure, and in most patients with essential hypertension these levels are not elevated (212). However, ETA and mixed ETA-ETB antagonists decrease blood pressure in mild to moderately hypertensive patients (128, 172). Furthermore, in patients with essential hypertension, but not in normotensive controls, the combined blockade of ETA and ETB receptors or, but to a lesser extent, the selective blockade of the ETA receptor per se increases the forearm blood flow and potentiates the endothelium-dependent vasodilatations to acetylcholine (26, 27, 71).

Endothelium-dependent vasodilatations to physical (shear stress) and/or pharmacological (acetylcholine, bradykinin, substance P, etc.) stimuli are impaired in the forearm, coronary, and renal vasculature as well as in various microcirculatory beds of patients with essential or secondary hypertension (143, 186, 242, 245, 246, 248). In patients with essential hypertension, but not in those with secondary (primary aldosteronism or renovascular hypertension) hypertension, nonspecific cyclooxygenase inhibitors partially restore the acetylcholine-induced forearm vasodilatation, suggesting in the former the release of EDCF (239, 248). By contrast, selective COX-2 inhibitors worsen the endothelium dysfunction in patients with essential hypertension (23), indicating that it is the COX-1 isoform that is associated with the generation of EDCF. In patients with coronary artery diseases, the impaired acetylcholine-induced forearm vasodilatation is restored by S 18886, a potent and specific antagonist of the TP receptor (10, 227), further substantiating that human endothelial cells synthesize cyclooxygenase derivatives acting as agonists at endoperoxide-thromboxane receptors. Inhibition of cyclooxygenase also enhances the bioavailability of NO because the vasoconstriction evoked by the administration of an inhibitor of NO synthase is restored (239). Vitamin C normalizes the impaired endothelium-dependent vasodilatation in the forearm and the coronary circulation in patients with essential hypertension, confirming that the generation of reactive oxygen species partially explains the decreased importance of NO (229, 240). Additionally, the production of NO can be impaired because of polymorphisms of the eNOS gene (202), deficiency in the essential cofactor tetrahydrobiopterin (90), impairment of the L-arginine transport system (215), and increased serum levels of asymmetric dimethyl-arginine (an endogenous inhibitor of NO synthase) (189, 250, 269).

In patients with essential hypertension, vasodilatations of the forearm vascular bed to bradykinin are resistant to an inhibitor of NO synthase. In the presence of this inhibitor they are of comparable amplitude in normotensive and hypertensive subjects (184, 185). In the latter, they become sensitive to ouabain, an inhibitor of Na+/K+ ATPase, suggesting that an uncharacterized EDHF-mediated component partially compensates for the reduced importance of NO (236).

In secondary hypertensive patients, the normalization of arterial blood pressure restores endothelium-dependent vasodilatations, indicating that the endothelial dysfunction is a consequence of the high arterial blood pressure (242). In human isolated arterioles, a transient increase in intravascular pressure directly and specifically impairs endothelium-dependent vasodilatation (183). However, in essential hypertension, endothelial dysfunction must precede the onset of the high arterial blood pressure because in young genetically predisposed normotensive offspring of essential hypertensive patients, the response to acetylcholine is already reduced (247). Furthermore, antihypertensive agents do not have the same impact on endothelial function despite similar reduction of arterial blood pressure (243, 259). Altogether the data available suggest that the endothelial dysfunction in essential hypertension is not directly related to blood pressure values and is probably genetically determined.

In patients with essential hypertension, diuretics given in monotherapy do not affect endothelial function (122, 166), and β-adrenergic blockers have minimal or even deleterious effects of endothelium-dependent vasodilatation (213, 238, 280a). Exceptions are nebivolol and carvedilol, possibly because of their NO donor and antioxidant properties, respectively (264). Calcium channel blockers, especially dihydropyridines, improve endothelial function, a phenomenon associated with a reduction in oxidative stress (238, 244). Additionally, certain dihydropyridines can evoke endothelium-dependent, NO-dependent relaxations, a property that is not linked to the calcium-blocking properties of these compounds (16, 277) and which involves bradykinin B2 receptor activation and/or eNOS phosphorylation (139, 301). In most of the studies involving acetylcholine-induced forearm vasodilatation, the angiotensin-converting enzyme inhibitors did not affect the response to acetylcholine (69, 91, 192, 241). However, these inhibitors improve endothelial function in subcutaneous and coronary arteries (6, 213). Antagonists of the angiotensin AT1 receptors produce similar beneficial effects on endothelial function as angiotensin-converting enzyme inhibitors but again do not affect the forearm vasodilatation evoked by the administration of muscarinic agonists (17, 71, 122, 214, 280a). However, in the forearm of essentially hypertensive patients, angiotensin-converting enzyme inhibitors logically potentiate both bradykinin-induced and flow-mediated vasodilatations (69, 241). Angiotensin-converting enzyme inhibitors and antagonists of angiotensin AT1 receptors reduce oxidative stress (69) and stimulate the release of NO (259).

Aortic stiffness has an independent predictive value for total and cardiovascular mortality, coronary morbidity and mortality, as well as for fatal stroke in patients with essential hyper-
tension (137). Pulse pressure and aortic stiffness are differently affected by antihypertensive drugs, possibly explaining why apparently equipotent antihypertensive agents do not show similar effects on endothelial function and on end-organ damage (12). Endothelial NO regulates arterial elasticity (120, 288), and an increase in pulse pressure is associated with an increase in endothelial oxidative stress and an impairment of endothelial function (206). In essentially hypertensive patients, the magnitude of the endothelium-dependent vasodilatation of the forearm is inversely correlated to the amplitude of pulse pressure (29) and the intimal-medial thickening of the carotid wall (70).

ENDOTHELIAL REGENERATION

Under physiological conditions, endothelial cells are normally quiescent and are among the most genetically stable cells of the body. They replicate at a very slow rate because their turnover time is over hundreds of days (84). However, during angiogenesis (59), pathological situations (hyperlipidemia, hypertension; 58, 218) and surgical interventions (angioplasty; 144, 223), endothelial cells can proliferate rapidly with a turnover time of less than 5 days. Nevertheless, the capacity of endothelial cells to divide is limited and ultimately the cells enter a state of growth arrest, i.e., senescence. Senescent endothelial cells are altered morphologically, have an increased generation of reactive oxygen species, have a decreased production of NO, and are more sensitive to apoptotic stimuli (18, 82, 159). Bone marrow-derived circulating endothelial progenitor cells can target denuded arteries and contribute to endothelial regeneration. The repair of vascular injury with the help of endothelial progenitor cells is associated with normalization of most, but not all, aspects of endothelial function (81). However, risk factors for coronary artery diseases are associated with impaired number and function of these endothelial progenitor cells (268). Altered vasomotion, which frequently occurs after coronary angioplasty and restenosis, also illustrates dysfunction of the endothelium (140).

Rat Carotid Artery

In the carotid artery of the rat after endothelial denudation by balloon angioplasty, the expression of eNOS is no longer detected immediately after injury (consistent with complete endothelial denudation), reappears after 2 days, and increases to preinjury levels after 14 days. These changes in eNOS expression are related inversely to the levels of expression of endothelin-1 and both ETA and ETB receptors (191). In this model, the endothelium-dependent relaxations are impaired and both the NO- and EDHF-mediated responses are blunted. However, the alteration in NO-dependent responses is transient and is restored 28 days after the procedure while the reduction of the EDHF-mediated responses is sustained (127, 145). The cell membrane of the smooth muscle is more depolarized in carotid arteries subjected to angioplasty, and endothelium-dependent hyperpolarizations are reduced (44). This dysfunction is associated with a decreased endothelial expression of both small and intermediate conductance Ca2+-activated channels (SKCα, and IKCα) (127).

The production of reactive oxygen species increases after injury. Thus gene transfer of SOD and catalase reduces restenosis and improves endothelial function (49). Early eNOS gene transfer inhibits the proliferation of vascular smooth muscle and reduces the neointimal vascular lesions after balloon injury (107, 280). In this model, numerous treatments have demonstrated a beneficial effect on neointimal proliferation and/or endothelium-dependent vasodilatations, including angiotensin-converting enzyme inhibitors, antagonists of the angiotensin receptors (116, 194, 221), endothelin-converting enzyme inhibitors, antagonists of endothelin receptors (32, 190), SOD mimetics (170), estrogen (286), and inhibitors of poly(ADP-ribose) polymerase-1 (300).

Porcine Coronary Artery

In pigs, 8 days after balloon endothelial denudation of the proximal portion of large coronary arteries, the entire denuded portion is covered with a regenerated endothelial lining with an apparent normal morphology. However, the following 3 wk endothelial cells continue to replicate and their phenotype is altered. The size of the endothelial cells is irregular, and giant multinucleated cells are observed (15, 60, 223). Such morphological alterations have been described in human arteries from aging and atherosclerotic patients and are indicative of the senescence of the endothelial cells (38). In segments of porcine coronary arteries with regenerated endothelium, the intimal cross-sectional area is already increased 4 wk after denudation (224). No lipid deposition is observed in food-restricted animals, but in those fed with a cholesterol-rich diet, atherosclerosis develops acutely in these denuded areas (226).

After endothelial regeneration, a progressive impairment of the endothelium-dependent responses is observed. One week after angioplasty, the endothelium-dependent relaxations appear normal. However, 3 wk later, segments of porcine coronary arteries with regenerated endothelium show an apparent selective impairment of Gt-mediated, endothelium-dependent relaxations, for instance, in response to serotonin and α2-adrenoceptor agonists (15, 224). The inhibition of the serotonin-induced, endothelium-dependent relaxation is associated with an impairment of the NO-dependent component of the relaxation, which progresses over time (60, 256, 271; Fig. 6). In this model, the decrease in NO production is associated with no change (60) or a decrease in endothelial NO synthase expression (54). However, regenerated endothelial cells show an accelerated incorporation of modified low-density lipoprotein followed by their intracellular oxidation (60, 118), indicating that the occurrence of oxidative stress exacerbates the decrease in NO bioavailability. The impairment of endothelial function and especially that of serotonin-induced, endothelium-dependent vasodilatation favors atherothrombosis. The reduced production of NO incapacitates the endothelium to resist adhesion and aggregation of platelets as well as the adhesion and transendothelial migration of leukocytes (162). Because at the site of denudation the endothelial cells are less responsive to serotonin, aggregating platelets produced vasoconstriction instead of vasodilatation, potentially exacerbating the coronary obstruction (223; Fig. 7). Repeated epicardial coronary artery endothelial injury induces downstream microvascular spasm and remodeling that involves generation of oxidative stress, activation of cyclooxygenase, and production of thromboxane A2 (3, 207), reminiscent of the production of an EDCF.

The endothelium-dependent relaxation to mediators for which the signal transduction does not involve Gi, such as...
bradykinin, is apparently not impaired at least during the first few weeks after denudation (224; Fig. 6). In response to bradykinin, the reduced NO availability is compensated by an increase in the contribution of the EDHF-mediated component (256, 257; Fig. 8). By contrast, both the NO- and the EDHF-mediated responses elicited by substance P or serotonin are reduced after angioplasty (256, 258). The different forms of endothelial dysfunction observed in response to various agonists must reflect the different mechanisms underlying the EDHF-mediated responses in the porcine coronary artery. The endothelium-dependent hyperpolarization evoked by substance P relies exclusively on the activation of endothelial Kᵦᵣ, whereas that produced by bradykinin involves both the activation of endothelial Kᵦᵣ and the release of cytochrome P450 metabolites (51, 285). In porcine coronary arteries with regenerated endothelium, the bradykinin-induced, endothelium-dependent relaxations and hyperpolarizations are preserved possibly because an increased production of EETs plays a compensatory role.

Fig. 6. Impairment of endothelium-dependent relaxation in porcine coronary artery with a regenerated endothelium (modified from Ref. 224). Top: serotonin 3 wk after endothelial denudation, segments of porcine coronary arteries with regenerated endothelium show a marked impairment of endothelium-dependent relaxation that persists over time. This impairment appears selective of Gₛ-mediated endothelium-dependent relaxations. Bottom: bradykinin. Endothelium-dependent relaxation to bradykinin is not affected in the first few weeks after endothelial denudation but become significantly impaired after 16 wk.

Fig. 7. Aggregating platelets and endothelium-dependent relaxation in native porcine coronary artery (LCX, left circumflex) and porcine coronary artery with a regenerated endothelium (LAD, left anterior descending) with and without endothelium (modified from Ref. 223). Left: control conditions (before denudation). In quiescent coronary arteries without endothelium, the aggregating platelets produce a contraction (2nd and 4th traces), whereas in coronary arteries with endothelium, aggregating platelets evoke endothelium-dependent relaxations of different amplitude depending on level of spontaneous myogenic tone developed by the isolated ring (1st and 3rd traces). Right: 4 weeks after endothelial denudation. In the LAD artery subjected to endothelial denudation, the endothelium-dependent relaxation followed by complete endothelial regeneration, the endothelium-dependent relaxation to aggregating platelets is no longer observed. In contrast, in the LCX artery, which has not been subjected to endothelial denudation, aggregating platelets still evoke endothelium-dependent relaxation. In healthy endothelial cells, the release of endothelium-derived relaxing factors prevents the constriction produced by aggregating platelets.
Antioxidant treatments prevent oxidative stress and, in injured epicardial coronary arteries, reduce neointimal formation and prevent restenosis (176, 217). Percutaneous eNOS gene transfer restores NO production in the injured artery and prevents luminal narrowing (272). A treatment with cervastatin in combination with the dihydropyridine nifedipine restores the eNOS expression and the endothelium-dependent relaxations attenuated by the balloon injury (54). Treatment with the angiotensin-converting enzyme inhibitor perindopril significantly inhibits neointimal formation and improves the NO-dependent component of endothelium-dependent relaxations (158). The latter beneficial effect has not been observed systematically with other angiotensin-converting enzyme inhibitors or antagonists of the angiotensin receptors (35, 97, 98, 133).

Human Coronary Artery

In patients subjected to percutaneous transluminal coronary angioplasty, the circulating levels of endothelin are increased, an effect attributed to the endothelial damage (61). Big endothelin-1 and endothelin-1 immunoreactivity is ubiquitous in atherosclerotic tissue, and endothelin-1 is released from these sites in response to mechanical stress (85). High circulating levels of endothelin-1 are associated with restenosis (249) and are a strong independent predictor of a major adverse clinical outcome in patients with primary coronary angioplasty (298).

Coronary artery segments subjected to balloon angioplasty are hyperactive to the vasospastic action of acetylcholine up to 6 mo following the intervention, indicating that the impairment of endothelium-dependent vasodilatation is unable to oppose the activation of the muscarinic receptors on the smooth muscle cells (121, 149, 275). The formation of isoprostanes, stable, free radical-catalyzed products of arachidonic acid and potent vasoconstrictors can be detected in the coronary sinus blood immediately after percutaneous transluminal coronary angioplasty, providing direct evidence for a local enhancement of oxidative stress (104). In addition to these locally and traumatically generated reactive oxygen species, coronary reperfusion contributes to the production of isoprostanes (198). Oxidized low-density lipoprotein levels are also elevated following percutaneous coronary intervention (263). Treatments with the antioxidant probucol or with HMG-CoA reductase inhibitors reduce clinical and angiographic restenosis after balloon coronary angioplasty (167, 253). The improvement of endothelial function induced by statins is positively correlated to therapy-induced change in serum oxidation susceptibility (168) and is most likely due to activation of endothelial NO synthase (268). By contrast, the effects of angiotensin-converting enzyme inhibitors in preventing restenosis have been generally deceptive, and this does not appear to be associated with insertion/deletion polymorphism of the gene encoding the angiotensin-converting enzyme (2, 124). The effect of antagonists of angiotensin receptors is uncertain (199).

CONCLUSIONS AND PERSPECTIVES

Hypertension, endothelial injury, as well as dyslipidemia (in particular hypercholesterolemia), diabetes, hyperhomocysteinemia, smoking, aging, and increased body mass index are major risk factors for the development of atherosclerosis. They are all associated with an increase in oxidative stress and endothelial dysfunction. However, depending on the pathology, the vascular bed studied, the stimulant, and these additional risk factors, the mechanisms underlying the endothelial dysfunction can be markedly different. A reduced bioavailability of NO, an alteration in the production of prostanoids, an impairment of endothelium-dependent hyperpolarizations, as well as an increased release of endothelin-1 can individually, or in association, contribute to endothelial dysfunction. Therapeutic interventions do not necessarily restore a proper endothelial function and, when they do, may improve only part of these variables.

Inflammation plays a key role in atherosclerosis and other cardiovascular diseases and is likely to be an important precursor of endothelial dysfunction (209, 254). In this respect, microparticles, small vesicles generated from circulating and vascular cells, could be one of the key factors linking inflammation, oxidative stress, apoptosis, and atherothrombosis. These circulating membrane fragments shed from the blebbing plasma membrane of activated or apoptotic cells are elevated under several pathological conditions (157), and their circulat-
ING LEVEL IS ASSOCIATED WITH ENDOTHELIAL DYSFUNCTION (5, 163, 284). THE PRECISE RELATIONSHIP BETWEEN INFLAMMATION AND ENDOTHELIAL DYSFUNCTIONS IS STILL POORLY UNDERSTOOD, AND THE EFFECTS OF THERAPEUTIC AGENTS ON THESE PARAMETERS REMAIN TO BE PROPERLY ASSESSED BOTH IN EXPERIMENTAL ANIMAL MODELS AND IN HUMAN DISEASE.

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