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Andreas J. Flammer, Thomas F. Lüscher





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Human endothelial dysfunction: EDRFs

Andreas J. Flammer · Thomas F. Lüscher

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Abstract Since the detection of nitric oxide two and a half decades ago, there has been an incredible boost in endothelial function research, which is fascinating the research community. Physiologically, endothelial cells synthesize a number of vasoactive substances. In particular, several endothelium-derived relaxing factors (EDRFs) have been characterized, whereby nitric oxide is the most important. In humans, endothelial dysfunction is one of the first clinically detectable alterations in the development of atherosclerosis and is characterized by an imbalance in the release of vasoactive substances. Thus, it is the aim of this article to give an overview about endothelial function in humans, to summarize the different possibilities to assess endothelial function in this species, and to give an overview of the role of EDRFs in different cardiovascular diseases.

Keywords Endothelium · Endothelium-derived relaxing factor (EDRF) · Nitric oxide · Prostacyclin · Endothelium-derived hyperpolarizing factor

Introduction

In the past, the endothelium was believed to be just a simple semipermeable membrane lining the inner part of arteries, veins, and lymphatic vessels. In the last three decades, however, thanks to extensive research in this field, it became apparent that this cellular monolayer is fundamental for the homeostasis of vascular tone (Fig. 1).

In humans, endothelial dysfunction is one of the first clinically detectable alterations in the development of atherosclerosis. In recent years, a number of studies assessing endothelial function have been performed in healthy subjects, as well as in patients. Through this research, the role of endothelial cells in health and cardiovascular disease could be largely defined. Clinical studies have been performed in several vascular beds, including the forearm vasculature, the microcirculation of different organs, as well as the coronary circulation. It is the aim of this article to summarize the different possibilities to assess endothelial function in humans and to give an overview of the role and alterations of endothelium-derived relaxing factors (EDRFs) in different cardiovascular diseases.

A. J. Flammer · T. F. Lüscher
Cardiovascular Center, Cardiology University Hospital Zurich,
Zurich, Switzerland

T. F. Lüscher
Institute of Physiology, Cardiovascular Research,
University Zürich-Irchel,
Zürich, Switzerland

T. F. Lüscher (✉)
Cardiology and Cardiovascular Physiology,
University Hospital Zurich,
Ramistrasse 100,
8091 Zurich, Switzerland
e-mail: cardiottfl@gmx.ch

Methods for assessing human endothelial dysfunction

Several methods have been developed in recent years; however, an optimal methodology does not exist so far, and hence, there is no clear gold standard. The different techniques used all have their advantages and disadvantages and allow for the investigation of different vascular beds.

Mainly large conduit arteries are able to dilate in response to reactive hyperemia (i.e., flow-mediated vasodilatation) or receptor-operated agonists such as acetylcholine

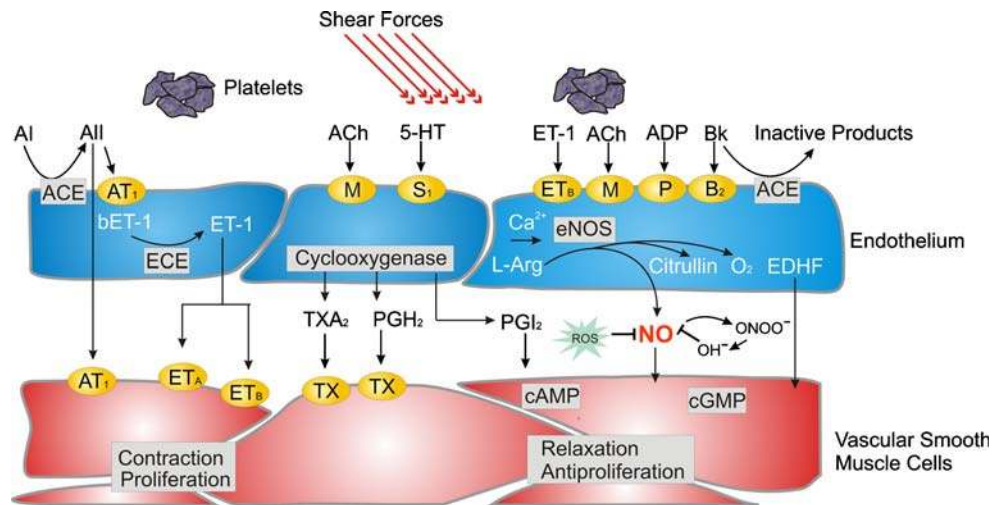


Fig. 1 Endothelium-derived vasoactive substances. Shear stress and activation of a variety of receptors leads to a release of nitric oxide (NO) by inducing endothelial nitric oxide synthase. It exerts relaxation of vascular smooth muscle cells and exerts antiproliferative effects as well as inhibits thrombocyte aggregation and leucocyte adhesion. Other endothelium-derived relaxing factors including endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (PGI_2) are also shown. ACE angiotensin-converting enzyme, ACh acetylcholine,

AI angiotensin I, AII angiotensin II, AT_1 angiotensin 1 receptor, Bk bradykinin, COX cyclooxygenase, ECE ET-converting enzyme, EDHF endothelium-derived hyperpolarizing factor, ET_A and ET_B endothelin A and B receptors, ET-1 endothelin-1, L-Arg L-arginine, PGH_2 prostaglandin H_2 , ROS reactive oxygen species, S_1 serotonergic receptor, TH thromboxane receptor, Thr thrombin, TXA_2 thromboxane, 5-HT serotonin

in the presence of an intact layer of endothelial cells. These responses are mediated by the release of nitric oxide (NO) or other vasodilator substances such as prostacyclin (PGI_2), able to activate smooth muscle cells (Fig. 1 and text below). Of note, not all blood vessels are able to dilate to the same extent in response to these stimuli, as the expression of specific endothelial receptors for these agonists as well as the intrinsic stiffness of a given blood vessel plays an important role. Nevertheless, independent by the technique used to assess function, a significant association between endothelial function and cardiovascular risk factors and disease has been noted. Indeed, in humans, endothelial dysfunction has been documented in the presence of most major cardiovascular risk factors including in arterial hypertension [59, 73], in normotensive individuals with a family history of hypertension [96], in active smokers [17, 116] and passive smokers [18], in dyslipidemia [15, 89], in aging subjects [59], in diabetes mellitus [13, 23, 62, 88, 91], in obesity [91], in hyperhomocysteinemia [105], and in patients with inflammatory or infectious diseases [36, 37, 48]. Moreover, a prognostic role of endothelial dysfunction of epicardial coronary arteries, as well as those of the peripheral circulation, has been documented [8, 40, 43, 45, 67, 81, 84, 85, 94, 104].

Flow-mediated vasodilatation:

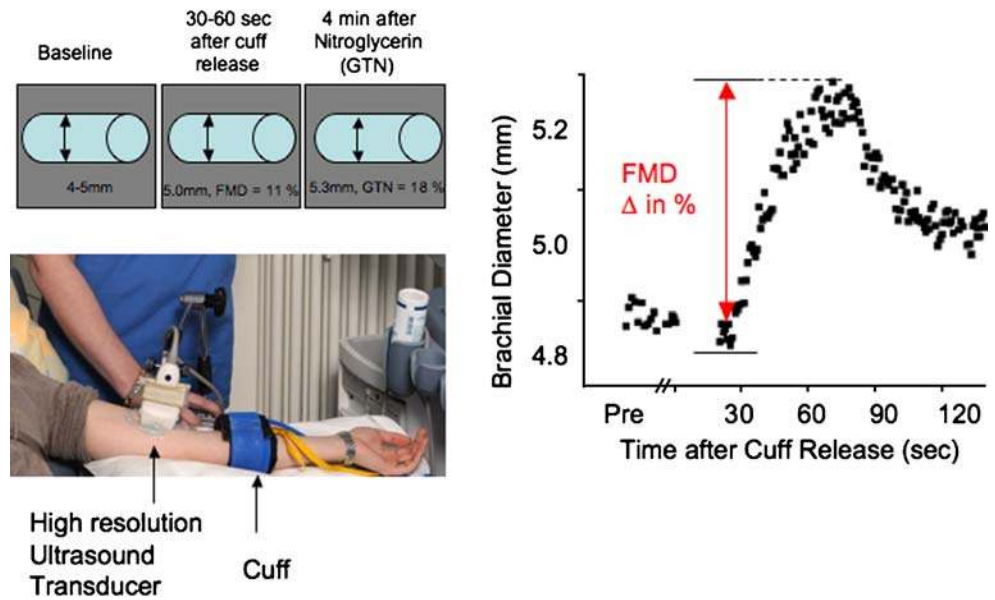
The most important and widely used techniques to assess endothelial function in humans take advantage of the fact that endothelial cells release NO and other endothelium-

derived relaxing factors in response to blood flow induced shear stress. To measure this response in vivo, Celermajer et al. developed an elegant noninvasive technique to determine flow-mediated vasodilatation (FMD) of the brachial or radial artery in response to reactive hyperemia after short periods of total blockage of blood flow by a blood pressure cuff [16]. Thereby, the change in diameter of the studied blood vessel is assessed by ultrasound at baseline and during reactive hyperemia (Fig. 2). This response is mainly NO-mediated as demonstrated by Joannides et al. [50, 51] (Fig. 3). Moreover, at least in patient populations, peripheral endothelial function as assessed by FMD correlates with coronary artery endothelial function [3]. The method, however, does require extensive training and experience and suffers standardization. Several attempts have been made to standardize the technique and the different protocols [20, 26].

Venous occlusion plethysmography

Forearm venous plethysmography is a more invasive technique, as it typically requires cannulation of the brachial artery and intraarterial administration of substances, hormones, or drugs. The technique essentially measures changes in volume of the forearm during brief periods of venous occlusion and thereby measures blood flow rather than changes in large artery diameter (see above). Substances including acetylcholine, bradykinin, and serotonin or nitroglycerin are usually infused to measure endothelium-dependent and endothelium-independent va-

Fig. 2 Flow-mediated vasodilatation. Schematic ultrasound images of the brachial artery at baseline, after reactive hyperemia induced flow-mediated vasodilatation and after nitroglycerin (GTN) application, are shown. Blood pressure cuffs can be placed on the upper or the lower side of the transducer in the antecubital fossa; however, the latter is the preferred method. On the left hand side, the time course of an FMD measurement is shown [20]. See text for further explanation



sodilatation, respectively (Fig. 4) [59, 73]. The advantage of this technique is the possibility to administer different agonists and antagonists and even novel substances at a systemically ineffective dose into the brachial artery, with the contralateral arm serving as a control. Changes in forearm blood flow as assessed by venous occlusion plethysmography are determined in both forearms, and results are expressed as the ratio of both arms. Although the microcirculation in the forearm is not a target organ of atherosclerosis, it seems that the response to acetylcholine has nevertheless an independent predictive value for future cardiovascular events [26].

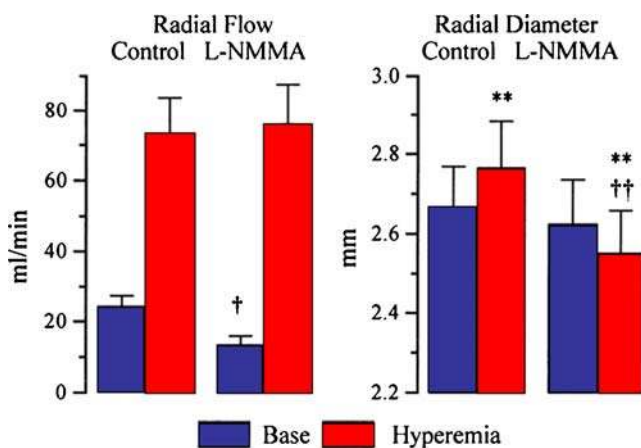


Fig. 3 NO in flow-mediated vasodilatation. Radial artery flow (milliliters per minute) and radial artery diameter (millimeters) measured at baseline and during reactive hyperemia before and after infusion of *NG*-monomethyl-L-arginine (*L-NMMA*). ** $P < .01$ vs base; $P < .05$ and $P < .01$ vs corresponding control value. Modified from Joannides et al. [50]

Coronary endothelial function measurements

Assessment of coronary endothelial function is always handicapped by its invasive nature. Indeed, it requires catheterization of usually the left coronary artery with an angiography catheter and the intracoronary infusion of acetylcholine, papaverine, or other substances such as L-monomethyl arginine and the assessment of changes in coronary artery diameter by quantitative angiography. However, if performed appropriately by experienced operators, it provides very valuable information about the coronary vascular bed. Indeed, the healthy coronary circulation with a functionally intact endothelium will respond to intracoronary acetylcholine infusion with epicardial and microvascular relaxation resulting in vasodilatation and an increase of coronary blood flow. However, if the endothelium is dysfunctional, acetylcholine induces paradoxical vasoconstriction and a decrease in coronary blood flow [60]. The response to intracoronary acetylcholine has important prognostic impact and predicts future cardiovascular events [84].

Finger plethysmography

Recently, a finger plethysmographic device allowing the detection of pulsatile arterial volume changes has been introduced [56, 58]. Similar to the assessment of endothelial function via the FMD technique by ultrasound of the brachial artery, a pressure cuff is placed on one upper arm, while the other arm serves as a control. After measuring baseline blood volume changes, the blood pressure cuff is inflated above systolic pressure and is then deflated to induce reactive hyperemia on one arm. Similar volume

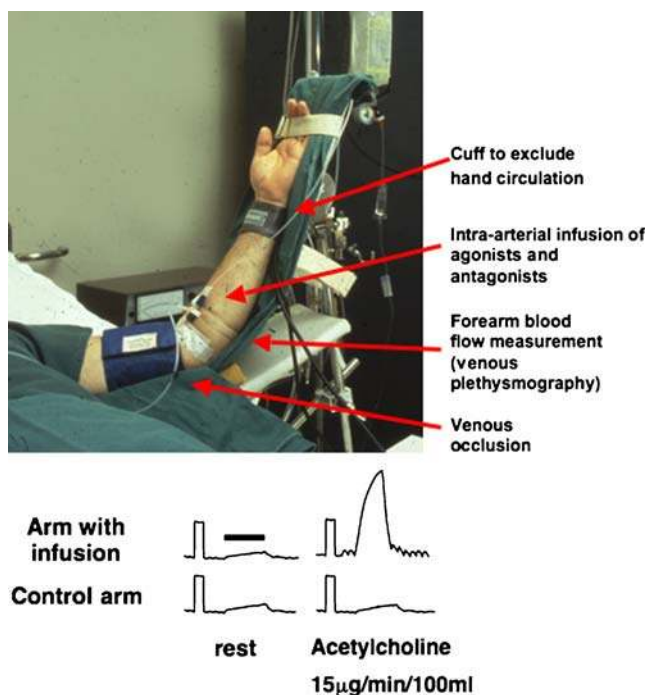


Fig. 4 Venous plethysmography. Demonstration of the strain-gauge venous plethysmography method to assess human peripheral endothelial microcirculatory function. The brachial artery is cannulated and substances including acetylcholine or nitroglycerin as well as drugs can be infused

changes after nitroglycerin can be measured. This technique tends to measure microvascular function.

Pulse wave analysis

With this noninvasive technique, the pulse-wave and velocity profile of the propagation of the arterial wave form and its reflected wave is assessed. The central aortic waveform is calculated as the augmentation index [115]. Although not the only contributor, endothelial function plays an important role in arterial stiffness and thus affects the results of this methodology as well. It therefore has been used to determine effects of endothelial mediators on arterial stiffness.

Endothelium-derived relaxing factors in human endothelial function

A considerable knowledge on the role of the endothelium in vascular homeostasis has accumulated as reflected by the contributions to this special issue on the endothelial saga. Physiologically, endothelial cells synthesize a number of antiatherogenic substances. In particular, several EDRFs have been characterized (Fig. 1). Most of them are released after an increase in intracellular calcium within endothelial

cells in response to shear forces and/or receptor-operated mediators. Currently, the most extensively studied molecules are NO, PGI₂, and endothelium-derived hyperpolarizing factors (EDHF; Fig. 1).

The contribution of these mediators to endothelium-dependent dilatation is inversely related to the vessel size. Indeed, NO- and PGI₂-mediated responses seem to be more important in conduit vessels, whereas EDHF seems to be more prominent in resistance arteries, particularly also in the coronary circulation [87].

Nitric Oxide

The term endothelium-derived relaxing factor was originally proposed by Robert Furchgott for a then unknown factor leading to relaxation of the smooth muscle of large arteries in response to acetylcholine. NO was later found to be the mediator of this response. NO is synthesized by the endothelial isoform of NO synthase (NOS) from its precursor L-arginine [71], which is inhibited by false substrates of the precursor of NOS, e.g., L-NG-monomethyl arginine (L-NMMA) [72]. NOS is a highly regulated protein and the endothelial isoform (eNOS) is predominantly found in endothelial cells. Its full function is dependent on activation of calmodulin and the presence of L-arginine and cofactors such as tetrahydrobiopterin (BH₄) [1, 92]. BH₄ supplementation increases NO synthesis in patients with hypercholesterolemia [24] and coronary artery disease [61].

NO is released from endothelial cells in response to activation of different receptors and especially to shear stress induced by blood flow (Fig. 1) [2, 39, 82]. The free radical has a very short half-life, easily crosses biological membranes and thus quickly diffuses from the endothelium to the vascular smooth muscle cell to activate soluble guanylyl cyclase which in turn induces an increase in cyclic guanosine monophosphate (cGMP) concentrations, thus leading to a relaxation of the smooth muscle cells with consequent vasodilatation (Fig. 1) [39, 70, 71, 90].

NO is a free radical which is scavenged for instance by reactive oxygen species (ROS) which play an important role in the pathogenesis of cardiovascular diseases. Rapid removal of ROS is important to protect cellular structures and NO from its inactivation. In many cardiovascular diseases, however, an increased oxidative stress is an important hallmark. For example, superoxide anion (O₂⁻), an oxygen radical, can scavenge NO to form peroxynitrite (ONOO⁻), which effectively reduces the bioavailability of endothelium-derived NO and causes posttranscriptional modification of proteins such as superoxide dismutase and prostacyclin synthase and DNA damage [83, 109]. In addition, O₂⁻ directly may act as a vasoconstrictor [4, 22, 52, 53]. A major source of O₂⁻ is the nicotinamide adenine

dinucleotide (NADH) dehydrogenase, a mitochondrial enzyme of the respiratory chain [110]. Its expression in human coronary arterial smooth muscle cells is upregulated by pulsatile stretch, thus generating increased oxidative stress [47]. O_2^- is finally degraded by superoxide dismutase (SOD), forming H_2O_2 which is further metabolized by catalase [38]. Unfortunately, the reaction between O_2^- and NO is three times faster than the detoxification of O_2^- by SOD [106]. Depending on the relative concentrations of NO and SOD, there may be a propensity for O_2^- to react preferentially with NO, resulting in decreased bioavailability of NO with its detrimental consequences (see above). Oxidative stress therefore not only eliminates protective NO but also leads to lipid peroxidation and endothelial cell death.

Importantly, despite its vasodilatation properties, NO has also antithrombogenic, antiproliferative, leukocyte adhesion-inhibiting effects, and influences myocardial contractility [2, 50, 51, 111]. Hence, NO has a very powerful antiathrogenic profile and endothelial dysfunction with decreased NO levels therefore favors vascular dysfunction and atherosclerotic disease.

The important role of NO in humans is illustrated best with its effect on arterial blood pressure. Of note, NO release is an important contributor to basal vascular tone also in humans. Thus, inhibition of NOS for instance with L-NMMA leads to an increase in arterial blood pressure [2, 71, 72, 82, 111].

NO in cardiovascular disease

With the above-mentioned techniques, endothelial function *in vivo* nowadays can be easily assessed in normal subjects as well as patients with cardiovascular risk factors or disease. Of interest, endothelium-dependent vasodilatation in response to acetylcholine is blunted in patients with hypertension, both in the forearm as well as in the coronary circulation [25, 46, 59, 73–77, 97–99] [31, 107]. Further, a reduced basal NO activity has been noted as the response to the NO inhibitor L-NMMA is significantly less in hypertensive patients compared with normotensive controls [12, 96]. Interestingly, normotensive offspring of hypertensive parents exhibit impaired endothelial function as assessed by the response to acetylcholine, and similar to patients with manifest hypertension, basal NO synthesis is already diminished [63]. Therefore, endothelial dysfunction in hypertension is at least in part caused by genetic factors and is not simply a consequence of the high arterial blood pressure [64]. Several genetic variations in the eNOS gene have been described [81]. Importantly, the diminished NO bioactivity is most likely due to an increase in ROS, which are able to scavenge NO [100].

In aging, the bioavailability of protective NO declines with a simultaneous increase in constricting factors. The

reason for the decline in NO in the elderly is not fully understood. Some studies suggest a decrease in the NOS activity in aging [5, 108], whereas others do not [112]. Likely, oxidative stress is responsible because of a prolonged exposure to ROS, with an increasing number in dysfunctional mitochondria in endothelial cells. Elderly humans who perform regular physical training are able to improve eNOS expression [102].

In active as well as passive smoking, a dose-related impairment in endothelial function due to a decreased eNOS activity has been demonstrated [68] [17, 18, 116]. Besides hypertension, aging, and smoking, there is evidence that all major cardiovascular risk factors (alone or in combination) blunt endothelial function by impairing NO synthesis or decreasing the bioavailability of the mediator at the vascular level by increasing oxidative stress or reducing the sensitivity of vascular smooth muscle to NO (for review, see [10]).

Therefore, many pharmacological as well as nonpharmacological interventions to decrease oxidative stress have been investigated. Acute administration of antioxidant vitamins (e.g., vitamin C or E) demonstrated amelioration of endothelial dysfunction in different conditions, e.g., in coronary heart disease [54]. However, long-term interventional studies did not show clinical benefits [19, 29]. Recently, other antioxidative compounds such as polyphenols, mainly found in plant-derived nutrition (for example in cocoa in high concentrations), have been shown to improve endothelial function not only by its antioxidative effects but also due to direct induction of NOS [21, 35]. Many pharmacological interventions have been shown to improve endothelial function due to different mechanisms; however, this would go far beyond the scope of this review [93].

Prostacyclin

Another important endothelium-derived relaxing factor which is released partly in response to shear stress as well as in response to acetylcholine is PGI_2 [55, 69, 78, 82]. PGI_2 is synthesized by cyclooxygenase (COX) from arachidonic acid [66] and increases cyclic adenosine monophosphate (AMP) in smooth muscle cells as well as in platelets. However, it seems that in contrast to NO, PGI_2 does not contribute to the maintenance of basal vascular tone of large conduit arteries [50]. Also, in the forearm circulation of humans, the response to acetylcholine is unaffected by aspirin suggesting that PGI_2 plays a minor role in the control of vascular tone. However, in patients with a decreased NO bioavailability as in atherosclerosis, COX-2-derived prostaglandins can play an important compensatory role [11, 95].

Moreover, PGI_2 exerts important platelet inhibitory effects. Indeed, NO and PGI_2 synergistically inhibit platelet activity [80]. Interestingly, when endothelial cells are

stimulated with agonists that increase intracellular calcium, NO is released continuously [44], whereas PGI₂ is released only in a transient manner [65].

In addition, PGI₂ facilitates the release of NO by endothelial cells [86], and conversely, the action of PGI₂ in the vascular smooth muscle is potentiated by NO and NO indirectly prolongs the half-life of cyclic AMP, the second messenger of prostacyclin [27].

Endothelium-derived hyperpolarizing factor(s)

EDHFs are molecules causing smooth muscle cells to hyperpolarize. Their involvement in the regulation of vascular tone is defined as the response that persists in the presence of combined inhibition of nitric oxide (by L-NMMA) and prostacyclin (by aspirin). They may play an important role as compensatory pathways for endothelium-dependent vasodilatation in the presence of reduced NO availability [101]. In hypertensive patients, for example, vasodilatation due to bradykinin is impaired because of NO alteration by oxidative stress, but is due to endothelium-dependent hyperpolarization. Thus, vasodilatation is significantly reduced by ouabain, a Na(+)/K(+)/ATPase inhibitor which blocks hyperpolarization [101]. However, studies examining the relevance of EDHF in humans are rather scarce, mainly due to the fact that commonly used inhibitors of the EDHF pathway are limited by their in vivo toxicity [6].

Studies in the animal have identified several molecules/mediators that might act as EDHF in different tissues and species [28]. Among them, K⁺ [30], cytochrome P450 metabolites [14, 57, 103], lipoxygenase products [33], NO itself [7], reactive oxygen species (H₂O₂) [32], cyclic adenosine monophosphate [79], C-type natriuretic peptide [114], and electrical coupling through myoendothelial gap junctions [41, 42]. Central to endothelium-dependent hyperpolarization is a potassium-mediated event with a reduction in intracellular K⁺ in vascular smooth muscle which can be triggered by all of above-mentioned mediators [34].

Carbon monoxide

Carbon monoxide (CO) is generated endogenously partly in the endothelium and may have similar effects as NO including relaxation of vascular smooth muscle cells [113] and antiproliferative actions [49], which are also mediated by an increased production of cGMP. It is, however, less powerful than NO. CO may protect endothelial cells against apoptosis because of its interaction with NF-kappaB [9]. Its role in human disease, however, is not yet clear.

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