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Endothelium-derived Vasoactive Factors and Hypertension: Possible Roles in Pathogenesis and as Treatment Targets

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Abstract Endothelial cells regulate vascular tone by releasing various contracting and relaxing factors including nitric oxide (NO), arachidonic acid metabolites (derived from cyclooxygenases, lipoxygenases, and cytochrome P450 monooxygenases), reactive oxygen species, and vasoactive peptides. Additionally, another pathway associated with the hyperpolarization of the underlying smooth muscle cells plays a predominant role in resistance arteries. Endothelial dysfunction is a multifaceted disorder, which has been associated with hypertension of diverse etiologies, involving not only alterations of the L-arginine NO-synthase–soluble guanylyl cyclase pathway but also reduced endothelium-dependent hyperpolarizations and enhanced production of contracting factors, particularly vasoconstrictor prostanoids. This brief review highlights these different endothelial pathways as potential drug targets for novel treatments in hypertension and the associated endothelial dysfunction and end-organ damage.

Keywords NO-synthase · Cyclooxygenase · Lipoxygenase · Cytochrome P450 Monooxygenase · NAD(P)H oxidase · Guanylyl cyclase · Potassium channels · Calcium channels · TRP channels · Gap junctions · Oxidative stress · Thromboxane A2 · Prostacyclin · Endothelial dysfunction · End-organ damage

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Introduction

Furchgott and Zawadzki [1] described the obligatory role of the endothelial cells during relaxations of the isolated rabbit aorta to acetylcholine and demonstrated the transfer of a vasodilator substance termed "endothelium-derived relaxing factor." This factor was identified as nitric oxide (NO) and became a major player in physiology, pharmacology, and pathophysiology [2]. Over the years, however, it became obvious that NO could not explain all endothelium-dependent responses, and that endothelium-dependent relaxations can be caused by hyperpolarizations of the underlying vascular smooth muscle cells that are not due to endothelium-derived NO but rather to endothelium-derived hyperpolarizing factors (EDHFs) [3]. Another important aspect of endothelium-dependent control of vasomotor tone emerged with the demonstrations that endothelial cells can produce contracting factors, particularly vasoconstrictor prostanoids [4, 5]. It has thus become obvious that no single factor or mechanism is responsible for all endothelium-dependent responses, and that their modulation by age and disease leads to endothelial dysfunction, which has become a hallmark of vascular disease and a predictor of major cardiovascular events [5]. This brief review presents some

recent information concerning the multiplicity of endothelium-dependent signals and their possible contribution to the control of vascular tone in health and its derangement in hypertension.

Endothelium-derived Nitric Oxide

A common denominator in hypertension, regardless of its etiology (essential, renovascular, malignant, or preeclamptic), is endothelial dysfunction, which involves reduced production, decreased bioavailability, and/or impaired cellular effects of NO [6]. Therefore, various potential therapeutic targets have been identified all along the L-arginine–NO-synthase–soluble guanylyl cyclase pathway (Fig. 1).

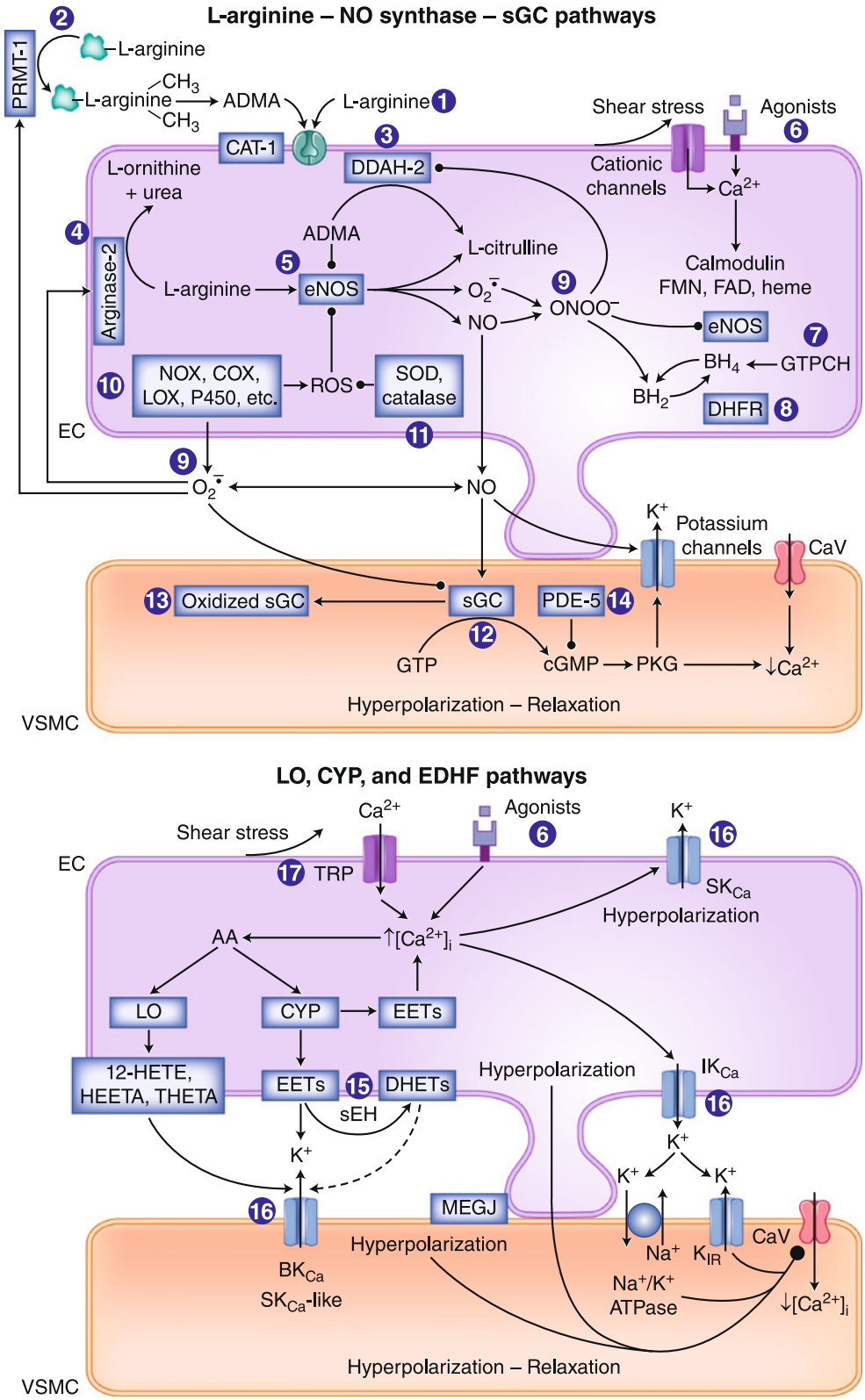
When **L-arginine** is deficient, endothelial NO synthase (eNOS) can generate both superoxide anions and NO, leading to the detrimental production of peroxynitrite. It is still a matter of debate whether L-arginine deficiency occurs in vivo to limit the production of NO by eNOS, but L-arginine supplementation improves endothelial dysfunction in hypercholesterolemia and hypertension [7]. In addition, endogenous analogues such as asymmetric dimethyl-L-arginine (ADMA) can compete with L-arginine for its specific membrane transporter and also directly for access to eNOS, where ADMA acts as an inhibitor. The plasma concentration of ADMA represents an independent predictor for all causes of cardiovascular mortality. Free dimethylarginines are the products of proteolytic degradation of arginine-methylated proteins by protein arginine N-methyltransferase type I (PRMT-I). In endothelial cells, ADMA is metabolized mainly by dimethylarginine dimethylaminohydrolase-2 (DDAH-2). During angiotensin II administration and oxidative stress, the observed elevation in ADMA levels is associated with an increase in the activity of PRMT and a decrease in the activity of DDAH. Silencing the DDAH-2 gene impairs endothelium-dependent relaxation and NO production. Therefore, the inhibition of PRMT-I and the activation or enhanced expression of DDAH-2 could be beneficial in treating cardiovascular disease [7].

Endothelial cells express **arginases** (with arginase-2 being the predominant isoform), which metabolize L-arginine to L-ornithine and urea. Arginase-2 competes with eNOS for substrate, and its expression and activity are enhanced in cardiovascular diseases, perhaps because of increased oxidative stress. In animal models, inhibition and gene deletion of arginase-2 improve endothelium-dependent relaxations and the vascular production of NO, prevent the development of hypertension, and decrease the generation of endothelial reactive oxygen species (ROS) and the formation of atherosclerotic plaques [8]. Arginase-2 may therefore represent a promising novel therapeutic target that could reverse vascular dysfunction in hypertension.

Fig. 1 Nitric oxide (NO) synthase, lipoxygenase (LO), and cytochrome P450 monooxygenase (CYP) pathways and responses mediated by endothelium-derived hyperpolarizing factors (EDHFs): potential sites of therapeutic intervention for hypertension. The *circled numbers* indicate potential sites of intervention: **1,** L-arginine supplementation. **2,** Inhibition of protein arginine N-methyltransferase type I (PRMT-I) to prevent the formation of asymmetric dimethyl-L-arginine (ADMA). **3,** Increased expression and/or activity of dimethylarginine dimethylaminohydrolase-2 (DDAH-2) to facilitate ADMA catabolism. **4,** Inhibition of arginase-2 to prevent L-arginine metabolism. **5,** Increased expression and/or activity of endothelial nitric oxide synthase (eNOS). **6,** Design of drugs that evoke endothelium-dependent relaxations. **7,** Enhanced expression and/or activity of guanosine triphosphate cyclohydrolase (GTPCH), the rate-limiting enzyme for tetrahydrobiopterin (BH_4) synthesis, or direct supplementation with BH_4 or its precursor sepiapterin. **8,** Enhanced expression and/or activity of dihydrofolate reductase (DHFR), involved in BH_4 regeneration. **9,** Scavengers of reactive oxygen species (ROS), antioxidants. **10,** Inhibition of the activity and/or expression of enzymes that generate ROS, such as NAD(P)H oxidases (NOX), cyclooxygenases (COX), lipoxygenases (LOX), or cytochrome P450 monooxygenases (P450). **11,** Enhanced expression and/or activity of enzymes that metabolize ROS, such as superoxide dismutase (SOD) or catalase (or, alternatively, synthesis of mimetics). **12,** Stimulation of soluble guanylyl cyclase (sGC). **13,** Activation of sGC. **14,** Inhibition of phosphodiesterase-5 (PDE-5). **15,** Inhibition of soluble epoxide hydrolase (sEH) to suppress degradation of epoxyeicosatrienoic acids (EETs). **16,** Opening calcium-activated potassium channels of small, intermediate, or large conductance (SK_{Ca}, IK_{Ca}, BK_{Ca}). **17,** Opening transient receptor potential channels (TRP). AA—arachidonic acid; BH_2—dihydrobiopterin; CAT-1—cationic amino acid transporters; CaV—voltage-activated calcium channel; cGMP—cyclic guanosine monophosphate; DHETs—dihydroxyeicosatrienoic acids, EC—endothelial cell; FAD—flavin adenine dinucleotide; FMN—flavin mononucleotide; HEETA—hydroxy-epoxyeicosatrienoic acid; 12-HETE—12-hydroxyeicosatetraenoic acid; K_{IR}—inward rectifying potassium channel; MEGJ—myoendothelial gap junction; $O_2^{\bullet-}$—superoxide anion; $ONOO^-$—peroxynitrite; PKG—protein kinase G; THETA, trihydroxyeicosatrienoic acid; VSMC—vascular smooth muscle cell

Reduced expression of **eNOS** could be responsible for decreased NO production, but in most situations where endothelial dysfunction is encountered, the expression of eNOS is increased paradoxically, most likely because oxidative stress generates hydrogen peroxide, which increases the expression of the enzyme. Endothelial dysfunction associated with this increased expression of eNOS shows that the ability to generate NO is reduced or its bioavailability is decreased. The reduction in NO generation can be attributed to eNOS uncoupling, whereby the enzyme itself is a source of superoxide anions and a cause of endothelial dysfunction [9].

Tetrahydrobiopterin (BH_4) is an essential cofactor that critically controls the assembly and activity of eNOS. Decreased endothelial levels of BH_4 are responsible not only for a reduction in NO production but also for the uncoupling of eNOS. BH_4 is highly susceptible to oxidation by peroxynitrite, forming BH_2 and ultimately biopterin. BH_4 is synthesized de novo from guanosine triphosphate



(GTP) following a series of enzymatic reactions, whereby GTP-cyclohydrolase I (GTPCH-I) is the first and rate-limiting step. An alternative pathway for BH_4 synthesis, the "salvage pathway," involves the formation of sepiapterin and its subsequent reduction in dihydrobiopterin (BH_2), which is further reduced by dihydrofolate reductase (DHFR) to form BH_4. Following oxidation, BH_4 can be regenerated by either DHFR or dihydropteridine reductase.

Increased vascular homocysteine is a risk factor for atherosclerosis; it leads to endothelial dysfunction, and some of its effects may be mediated by inhibition of BH_4 de novo synthesis [10]. Direct supplementation with BH_4 or its precursor, sepiapterin, improves endothelial function in animals, in smokers, and in patients with hypertension, diabetes, hypercholesterolemia, or coronary disease. Enhancing the expression or the activity of GTPCH-I (for de novo BH_4 synthesis) or of DHFR (for BH_4 regeneration) may prevent the occurrence of endothelial dysfunction. Alternative strategies include supplementation with folic acid, which enhances the binding affinity of BH_4 to NOS, stabilizes BH_4 chemically, and stimulates DHFR. Preventing peroxynitrite formation (and therefore BH_4 oxidation) or facilitating the recycling of BH_4 regeneration with vitamin C can also be considered [9].

Inhibition or deletion of eNOS causes accelerated atherosclerosis in rabbits and mice. However, apoE$^{-/-}$ mice overexpressing eNOS develop larger atherosclerotic lesions than control apoE$^{-/-}$ mice. Again, this paradox is explained best by the peroxynitrite-dependent **uncoupling of eNOS** and the subsequent production of superoxide anions. However, eNOS uncoupling can be prevented if the upregulation of eNOS is associated with an increased availability of essential cofactors such as BH_4. Compounds such as AVE9488 and AVE3085, which enhance eNOS promoter activity, possess such a coordinated activity and validate the concept that enhanced transcriptional expression of eNOS can be beneficial in preventing cardiovascular diseases [11•].

Besides preventing eNOS uncoupling, other therapeutic strategies can be designed to restore proper NO levels. These include drugs that stimulate the release of NO by endothelial eNOS, NO donor drugs, antioxidant compounds, drugs that boost the antioxidant defense mechanisms, and inhibitors of enzymes involved in the generation of ROS.

Some antihypertensive drugs—for instance, nebivolol, various dihydropyridines, angiotensin-converting enzyme (ACE) inhibitors, angiotensin (AT_1) receptor blockers (ARBs), and possibly renin inhibitors—can directly or indirectly stimulate the release of endothelial NO. Additionally, chronic treatments with ACE inhibitors, ARBs, or renin inhibitors increase eNOS expression. Although chronic treatment with ACE inhibitors or ARBs consistently improves endothelial function in animal models of hypertension, variable effects have been reported in hypertensive patients, possibly because of the multifactorial etiology of essential hypertension or differences in the experimental clinical protocols [12].

Besides uncoupled eNOS, **NAD(P)H oxidase** is a predominant source of excess ROS in the vascular wall in essential hypertension [13]. Thus, inactivation of superoxide anions with superoxide dismutase mimetics or scavenging of ROS with antioxidants seems an obvious way of increasing NO bioavailability. Such strategies have been successful in various animal models of hypertension. However, large clinical trials of chronic antioxidant therapy in hypertensive patients have not produced major reductions in arterial blood pressure and did not improve the associated morbidity and mortality, with the possible exception of chronic intake of polyphenols (present in red wine, fruit, and vegetables) [14]. The reasons underlying these failures are not clear. Synthetic inhibitors of NAD(P)H oxidase have been proposed as an alternative, but it is still uncertain which isoforms of NAD(P)H should be inhibited, and the available inhibitors are neither specific nor potent enough, and they have poor pharmacokinetic properties [15••].

Activation of the **renin-angiotensin system** with subsequent stimulation of AT_1 receptors is a major stimulus for NAD(P)H oxidase and production of ROS in both animal models and in hypertensive patients [6]. Preventing ROS generation—by deleting a subunit of NAD(P)H oxidase, for instance—causes resistance to angiotensin II–induced hypertension and markedly reduces the associated endothelial generation of superoxide anions [16]. In rat models of hypertension, ACE inhibitors and ARBs decrease the generation of superoxide anions, diminish the amplitude of endothelium-dependent contractions, and restore the amplitude of both NO-mediated and EDHF-mediated endothelium-dependent relaxations [17]. In addition to lowering lipids, statins also decrease the expression of NAD(P)H subunits and increase eNOS expression, improving the balance between NO and ROS. These endothelial effects may contribute to the pleiotropic effects of these compounds.

To date, the clinical use of **NO donor drugs** is limited by the development of nitrate tolerance, a complex multifactorial phenomenon associated with the generation of oxidative stress and endothelial dysfunction [18]. New chemical classes of NO donors, some of which can spontaneously release NO, have been synthesized and may have a therapeutic interest beyond the treatment of coronary disease and heart failure. Hybrid compounds possessing dual activity also have been synthesized. Earlier compounds such as nicorandil (an organic nitrate that opens potassium channels) and nipradilol (a nonselective adrenoceptor blocker with NO-releasing properties) are still predominantly prescribed for angina and glaucoma, respectively. New hybrid compounds including NO-releasing antiadrenergic drugs, NO-releasing dihydropyridines, NO-releasing statins, NO-releasing ARBs, and NO-releasing ACE inhibitors are of therapeutic interest for treating hypertension and associated end-organ damage [19•].

The regulation of **nitric oxide bioactivity** can also be achieved by modulating soluble guanylyl cyclase, the main physiological target of NO, or the half-life of the signaling molecule, cGMP, which is readily hydrolyzed by phosphodiesterases. Two different classes of compounds—stimulators and activators—interact with **soluble guanylyl cyclase** [20•, 21•]. The former stimulate the enzyme in an NO-independent but heme-dependent manner; acting as allosteric modulators, they enhance NO-dependent cGMP production. By contrast, activators of soluble guanylyl cyclase target the enzyme when its heme is oxidized and no longer responsive to NO. Both activators and stimulators of soluble guanylyl cyclase are potent vasodilators and inhibitors of platelet aggregation. The stimulators are currently undergoing clinical trials in acute decompensated heart failure and peripheral artery occlusive disease, and the activators are being evaluated in pulmonary hypertension. Potential therapeutic indications of these new compounds include hypertension, myocardial ischemia, erectile dysfunction, atherosclerosis, and thrombosis [20•, 21•].

Phosphodiesterase 5 (PDE-5) was the first identified selective cGMP esterase in the cardiovascular system and is the major isoform involved in the hydrolysis of the cGMP pools generated by the activation of soluble guanylyl cyclase. Specific PDE-5 inhibitors are currently prescribed for erectile dysfunction but, in the future, their therapeutic indications may also include pulmonary hypertension, heart failure, and essential hypertension [22].

Endothelium-dependent Hyperpolarizations

EDHF-mediated responses can provide a vasodilator reserve in hypertension and can compensate, at least temporarily, for endothelial dysfunction due to compromised synthesis or availability of NO [3]. However, reduced endothelium-dependent hyperpolarizations also can contribute to arterial dysfunction in animal and human cardiovascular disease, especially hypertension and diabetes [3]. In any case, whether EDHF-mediated responses are diminished or conserved, it is still possible to pharmacologically stimulate the endothelial cells in order to inhibit the underlying vascular smooth muscle, thereby improving vasodilator responses and lowering arterial blood pressure in experimental and human hypertension or reversing endothelial dysfunction in diabetes [23, 24, 25••]. There are presumably a variety of structurally very distinct factors or pathways that can produce **EDHF-mediated dilatations**. Indeed, residual NO, products of the metabolism of arachidonic acid (including prostacyclin), hydrogen peroxide, potassium ions, and C-natriuretic peptide all have been proposed as EDHFs. In addition, a nonchemical EDHF-signaling pathway involves transfer of negative charges from the endothelium to the smooth muscle via gap junctions [3]. In the case of EDHF pathways involving K^+ ions and electrical gap-junctional myoendothelial coupling, activation of endothelial calcium-activated potassium (K_{Ca}) channels initiates the dilator response in many preparations by triggering hyperpolarization and K^+ release (Fig. 1). The in vivo relevance of endothelial K_{Ca} channels is indicated by the severely impaired EDHF-mediated responses and the elevated arterial blood pressure in mice deficient of endothelial intermediate-conductance channels (IK_{Ca}, KCa3.1) and small-conductance channels (SK_{Ca}, KCa2.3) [24, 26••]. Moreover, selective openers of endothelial IK_{Ca} and SK_{Ca}, NS-309 [23] and SKA-31 [27], may be of therapeutic use in cardiovascular disease because they have been shown to improve endothelial function in type 2 diabetes [23] and to lower blood pressure in mice with angiotensin II–induced hypertension [27].

Hydrogen peroxide, another putative EDHF, may amplify this IK_{Ca}- and SK_{Ca}-driven EDHF system by facilitating inositol-trisphosphate (IP_3)-mediated Ca^{2+} release and thus enhancing endothelial Ca^{2+} signaling [3, 28], thereby further stimulating the two channels. However, the well-known harmful effects of hydrogen peroxide make it questionable whether pharmacologic enhancement of its production could be a strategy to lower arterial blood pressure [29].

Of the products of the metabolism of arachidonic acid, **eicosanoids** generated by lipoxygenase (LO) and cytochrome-P450 (CYP) monooxygenase pathways are the most likely candidates as diffusible endothelium-derived relaxing factors [30•]. LO and CYP pathways are complex and yield many metabolites with diverse effects on vascular tone. The mechanisms by which LO-derived and CYP-derived eicosanoids produce vasodilatation include the activation of potassium channels (Ca^{2+}-activated large-conductance [BK_{Ca}] channels and small-conductance [SK_{Ca}-like] channels) leading to hyperpolarization, closure of L-type Ca^{2+} channels, and thus relaxation (Fig. 1). Because of this ability of LO-derived and CYP-derived eicosanoids to both hyperpolarize and relax smooth muscle, their vasodilator activity has been linked to EDHF-mediated responses in several vascular beds [3, 30•].

The endothelium expresses the three major LO isoforms: 5-LO, 12-LO, and 15-LO. The products of 12-LO and 15-LO cause dilatation, whereas leukotrienes generated by 5-LO are associated with vasoconstriction [30•]. The 12-LO product, 12(S)-HETE, produces vasodilatation by activating BK_{Ca} channels and thus hyperpolarizing the vascular smooth muscle cells, whereas HEETA and THETA, generated by 15-LO, open SK_{Ca}-like channels [30•]. The role in vivo of these LO products as endothelium-derived relaxing factors is uncertain. Pharmacologic inhibition with BW755C or genetic knockdown of 12/15-LO had no effect on arterial blood pressure [30•], but gene transfer of human

15-LO-1 enhanced the hypotension caused by acetylcholine [30•]. In contrast, LO inhibitors reduce angiotensin-II–induced hypertension [30•], and inhibition of 12/15-LO by baicalein and derivatives has beneficial effects in experimental ischemic stroke [30•]. Considering the blood pressure lowering effects and cerebrovascular protective effects of LO inhibitors on one hand, and the beneficial effects on arterial blood pressure of 15-LO overexpression on the other hand, it is difficult to decide on the potential benefit of stimulating the production of LO-derived endothelium-derived relaxing factors in hypertension. With respect to inflammatory responses and atherosclerosis, the polymorphisms in the human 12/15-LO gene (*ALOX15*) lead to either enhanced enzyme activity with a lowered risk of coronary artery disease [31] or to impaired enzyme activity with enhanced risk [32].

Endothelial cells express **CYP**s of the 4A, 2C and 2J subfamilies [3, 30•]. CYP4A ω-hydrolase produces the vasoconstrictor 20-HETE, but CYP2C and CYPJ2 epoxygenases generate epoxyeicosatrienoic acids (EETs)—5,6-EET, 11,12-EET, or 14,15-EET—all of which produce vasodilatation in certain human arteries [3, 30•]. Like other arachidonic acid metabolites, their vasodilator action is closely related to EDHF-mediated arteriolar responses because they stimulate BK channels and cause hyperpolarizations followed by relaxation [3]. Moreover, they also facilitate endothelial Ca^{2+} signaling and likely augment NO synthesis in an autocrine fashion [33]. In addition, 5,6 EET, 11,12-EET, and 14,15-EET, as well as their degradation products, dihydroxyeicosatrienoic acids (DHETs), may produce vasodilatation in a strictly NO-dependent fashion [33]. This would be explained if EETs/DHETs stimulate NO formation, either by causing eNOS phosphorylation or by enhancing Ca^{2+} signaling following activation of cation channels of the transient receptor potential gene family (*TRPV4*) [24, 29]. Whether these vascular **TRPV4** channels can be treated with drugs is uncertain because of potential deleterious effects [24, 29]. Importantly, the CYP pathway must be relevant also for hypertension: clinical data have demonstrated the ability of CYP products to compensate at least in part for the loss of NO-mediated dilator responses in patients with essential hypertension, and soluble epoxide hydrolase (sEH), an inhibitor of the EET degrading enzyme, exerts blood pressure–lowering effects in experimental hypertension involving the activation of the renin-angiotensin system [25]. This blood pressure–lowering effect was not seen after inhibition of NO synthesis by L-arginine-methylester (L-NAME), however [33], thus further showing that augmented EET levels favor NO formation, which by itself could lower arterial blood pressure. A specific inhibitor of sEH, AR9281 [25], has been tested in clinical trials for antihypertensive efficacy and for the treatment of type 2 diabetes but has failed because of lack of efficacy. Nevertheless, data from animal studies show that sEH inhibition or gene deletion could be effective in pulmonary hypertension or atherosclerosis and may have protective effects on brain, heart, and kidneys [25, 34]. The relevance of CYPs and sEH for cardiovascular disease is supported further by the identification of polymorphisms (causing reduced enzyme activities), which have been associated—although not consistently—with a higher risk for cardiovascular disease, hypertension, and type 2 diabetes [35]. Considering BK channels as putative targets of the products of both CYP and 15-LO, it is noteworthy that "gain of function" polymorphisms in BK-subunit genes decrease the risk of hypertension, whereas "loss of function" polymorphisms increase the risk [36].

Endothelium-dependent Contractions

With aging and in various cardiovascular diseases—particularly essential (spontaneous) hypertension—endothelial dysfunction is importantly due to the release of endothelium-derived contracting factors (EDCFs) that counteract the effect of endothelium-derived vasodilators. In healthy blood vessels, the release of EDCFs is tempered by the presence of NO [37] and EDHFs [38]. The major EDCFs that contribute to endothelial dysfunction in hypertension appear to be products of the metabolism of **arachidonic acid**. This conclusion is based mainly on data obtained in arteries of the spontaneously hypertensive rat (SHR), although it is supported by indirect evidence in humans [4, 5, 39].

Upon activation of the endothelial cell and the resulting increase in cytosolic **Ca^{2+} concentration**, arachidonic acid is released from cell membrane phospholipids, whereby phospholipase A_2 (PLA_2), in both calcium-dependent and calcium-independent forms, contributes to the process. Actually, the activation of calcium-dependent PLA_2 explains the calcium dependency of endothelium-dependent contractions [40•, 41]. Procedures that reduce the increase in endothelial Ca^{2+} concentration, such as the administration of vitamin D_3, curtail the occurrence of endothelium-dependent contractions [42].

The released arachidonic acid is then metabolized by **cyclooxygenases** (COX), an enzyme with two major isoforms: COX-1 is expressed constitutively and is usually abundant in all animal and human endothelial cells, whereas endothelial COX-2 is induced mainly during inflammatory responses in nearly all animals. In humans, although COX-2 is the predominant contributor to the systemic generation of prostacyclin, endothelial COX-1 is also a major source of prostaglandins in both healthy and diseased blood vessels [43–46]. In arteries of the SHR, there is a marked increase in

the mRNA expression and protein presence of COX-1, which underlies the augmented release of EDCFs [43].

The formed unstable endoperoxide (prostaglandin H_2, PGH_2) is metabolized further into prostaglandin D_2, E_2, $F_{2\alpha}$, I_2 (prostacyclin), and thromboxane A_2 by specific synthases. Depending on the blood vessel studied and the agonist used to activate the endothelial cells, different prostaglandins can contribute to endothelium-dependent contractions. In the SHR aorta, PGH_2 and prostacyclin appear to play the dominant role, mainly because of the abundance in the endothelial cells of prostacyclin synthase, compared with the other specific synthases, resulting in the overwhelming production and release of **prostacyclin** [43, 47]. The contribution of prostacyclin to EDCF-mediated responses may seem paradoxical, as one intuitively would expect the prostanoid to contribute instead to endothelium-dependent relaxations. Indeed, "specific" prostacyclin IP receptors are predominantly coupled to the Gs-adenylyl-cyclase-cyclic adenosine monophosphate (cAMP)-PKA pathway, and their activation results in vasodilatation. Patients with a dysfunctional IP receptor mutation show accelerated atherothrombosis [48••]. A characteristic of the SHR (and of aged normotensive rats) is that their vascular smooth muscle cells have lost responsiveness to IP receptor activation [4, 39].

EDCFs diffuse to the underlying smooth muscle and cause contraction. When they reach their target, prostaglandins interact preferentially, but not exclusively, with specific seven transmembrane domains, G-protein-coupled receptors (DP, EP, FP, IP, and TP receptors). Of all the prostanoid receptors leading to activation of vascular smooth muscle cells, **TP receptors** appear to be solely responsible for endothelium-dependent contractions. This conclusion is based on numerous observations that TP receptor antagonists abolish EDCF-mediated responses [39, 43, 45, 47]. Thromboxane A_2 is by far the preferential physiological ligand of TP receptors, but endoperoxides (PGH_2) and higher concentrations of the other prostaglandins (including prostacyclin, especially in vascular smooth muscle lacking IP receptor sensitivity), as well as isoprostanes, activate this receptor with a varying range of potency. Hence, at least in the SHR, endoperoxides and prostacyclin play a key role as EDCFs [39, 43]. To judge from data obtained in the SHR aorta, the TP receptors in this strain are hyperresponsive to endogenous vasoconstrictor prostanoids early in the hypertensive process. This hyperresponsiveness, the IP receptor insensitivity, and the larger release of endoperoxides and prostacyclin then explain the preponderance of endothelium-dependent contractions and the resulting endothelial dysfunction in the blood vessels (Fig. 2).

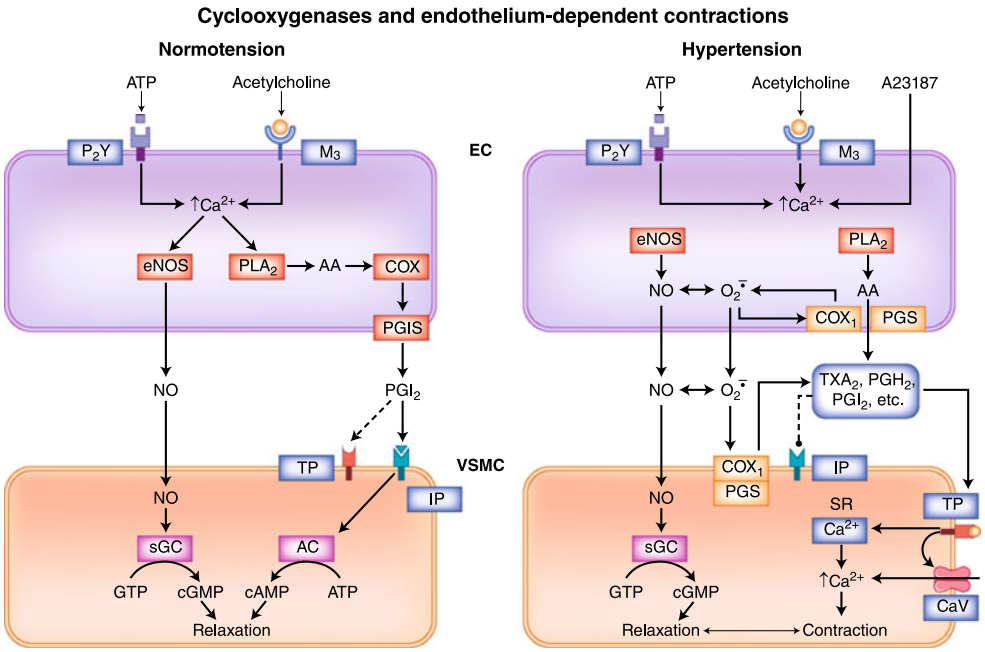


Fig. 2 Endothelium-dependent effects of acetylcholine in aorta of Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). **Left,** Endothelium-dependent relaxations in normotensive WKY rats. **Right,** Cyclooxygenase-dependent, endothelium-dependent contractions to acetylcholine in SHR aorta. A23187—calcium ionophore; AA—arachidonic acid; AC—adenylyl cyclase; ATP—adenosine triphosphate; cAMP—cyclic adenosine monophosphate; CaV—voltage-activated calcium channel; cGMP—cyclic guanosine monophosphate; COX_1—cyclooxygenase 1; eNOS—endothelial nitric oxide synthase; GTP—guanosine triphosphate; IP—PGI_2 receptor; M—muscarinic receptor; $O_2^{\bullet-}$—superoxide anion; PGH_2—endoperoxide; PGI_2—prostacyclin; PGIS—prostacyclin synthase; PLA_2—phospholipase A_2; P_2Y—putinergic receptor Y_2; sGC—soluble guanylyl cyclase; SR—sarcoplasmic reticulum; TP—TP receptor; TXA_2—thromboxane A_2. (*From* Félétou and Vanhoutte [17]. Used with permission of The American Physiological Society.)

TP receptor antagonists given in vivo evoke no change or only minor changes in arterial blood pressure, but they limit the endothelial dysfunction associated with hypertension, diabetes, and atherosclerosis; they are potent antithrombotic agents; and they reduce vascular inflammation. Therefore, TP receptor antagonists possess a unique potential to treat cardiovascular complications, exerting therapeutic benefits beyond those associated with COX inhibition only [39]. Actually, the addition of TP antagonism to COX-2 inhibition activity may improve the cardiovascular risk profile of COX-2 inhibitors [46].

Prostaglandin $F_{2\alpha}$ is also a potent vasoconstrictor; when administered in mice, it increases arterial blood pressure. In the hamster aorta, it mediates endothelium-dependent contractions [45]. Genetic deletion of the FP receptor, its preferential receptor, reduces blood pressure, and it delays the occurrence of atherosclerosis in low-density-lipoprotein (LDL) receptor–deficient mice. Blockade of FP receptors may offer a new approach to treating hypertension and its associated vascular complications [49].

Conclusions

Endothelial dysfunction, a hallmark of the hypertensive blood vessel wall, probably reflects the premature aging of the intima exposed to the chronic increase in arterial blood pressure. It is caused by complex changes in the balance between endothelium-dependent vasodilator and vasoconstrictor signals. The release of NO and the occurrence of endothelium-dependent hyperpolarizations can be endangered. As a result, the production of vasoconstrictor prostanoids (mainly endoperoxides and prostacyclin) is unleashed. Augmenting the bioavailability of NO, favoring the occurrence of EDHF-mediated responses, and preventing the release or action of EDCFs all can contribute to restoring proper endothelial function and thus can delay the appearance of vascular complications resulting from the hypertensive process.

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