Oxidative Stress Theory of Hypertension

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Edited by

Ramón Rodrigo

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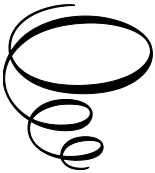

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PREFACE

Hypertension is a major independent and progressive risk factor for cardiovascular disease. It still remains a leading cause of morbidity and mortality worldwide and the biggest contributor to global mortality accounting for around 10.4 million deaths annually, according to recent data provided by the World Health Organization. Unfortunately, being asymptomatic, about one-third of patients are unaware of having this condition, remaining untreated and thus increasing the risk of stroke and heart disease. These characteristics have contributed to this pathology being referred to as a silent killer. When this disease remains chronically untreated, it gives rise to the development of complications often referred as target-organ damage—mainly kidney, heart, and brain— damage that is often not reversible. Consequently, the causes of death in hypertensive patients include heart failure, end-stage chronic renal disease, or stroke.

Most causes of hypertension are not identifiable, being a multifactorial development and referred to as primary or essential hypertension, accounting for more than 90% of cases. The rest of hypertensive patients have identifiable factors that cause secondary hypertension, with a known direct cause such as kidney disease or endocrine derangements, among others. The pathophysiological mechanism of hypertension has not been well elucidated; however, reactive oxygen species appears to be involved in both classes of hypertension, having a causal role in this pathology. Indeed, reactive oxygen species are mediators of hormones causing vasoconstriction in the physiological conditions; however, when the antioxidant defense system is overwhelmed by the production of these species, oxidative stress occurs and participates in triggering pathophysiological cascades. Consequently, a functional impairment ensues in the vascular wall leading to the development of endothelial dysfunction, followed by structural changes including inflammation, atherosclerosis, or fibrosis, among others. It is noteworthy that oxidative stress is involved in each one of the stages of this pathological sequence of events. Thus, it is reasonable to consider the therapeutic role of antioxidants in reducing the functional and structural oxidative damage caused by increased reactive oxygen species. However, human studies have shown inconsistent results when using antioxidants as therapy for hypertension. Despite this, most of the antihypertensive drugs currently in use have antioxidant properties. Therefore, it is reasonable to

propose that natural exogenous antioxidant supplements are candidates to be tested, based on their ability to contribute in a synergistic effect with antihypertensive drugs, especially when the latter are recognized to evoke a suboptimal therapeutic response in hypertensive patients. Unfortunately, the treatment of hypertension is aimed at lowering blood pressure rather than against the causal agent. Indeed, it is known that despite the numerous drugs available as pharmacological resources for the treatment of hypertension, and the need to indicate multidrug regimens, treatment-resistant hypertension is present with a prevalence between 11% and 21% of cases and has more than doubled during the last 25 years. Furthermore, with recent findings demonstrating a decreased incidence of cardiovascular events when applying strict goals for systolic blood pressure, usually in the range of 120 and 140 mmHg, it is reasonable to assume that future clinical interest will remain centered in blood pressure lowering drugs.

The inconsistency between human studies and experimental protocols in showing the association of hypertension and oxidative stress biomarkers indicates that studies are still lacking a complete knowledge of the pathophysiology behind oxidative stress-dependent hypertension. The aim of this book is to present available data related to the pathogenic mechanisms accounting for the role of oxidative stress in the induction, development, and maintenance of human arterial hypertension. In addition, the contribution of antioxidants as therapeutic agents to treat or prevent hypertension is also discussed as part of the paradigm presented here.

In order to reach our objective, the following chapters are presented:

1. **Role of the endothelium in vascular homeostasis**. Endothelial cells play a key role in the response of the vascular wall to local and systemic stimulus. Thus inflammation, hemostasis, angiogenesis, and vascular tone are modulated by mediators of these cells. Among these functions, the modulation of vasomotor tone is particularly sensitive to the intracellular redox balance occurring in endothelial and vascular smooth muscle cells. This balance, in turn, determines the predominance between vasoconstriction vs. vasodilation, mainly increasing blood pressure through cell signaling effects of ROS and oxidative stress.

2. **Involvement of ROS in blood pressure modulation**. The generation of oxidative stress induces blood pressure effects not solely due to the direct biological actions of ROS on the vascular wall, but also at the level of central nervous system (rostral ventrolateral medulla) and juxtaglomerular apparatus. In turn, renin

release leads to angiotensin II production, thereby inducing NADPH activation, an event also resulting from the effects of other hormones such as endothelin-1 and urotensin II, among others, all of which should exert actions responsive to antioxidants; however, clinical data remains controversial and new studies are needed. The involvement of peptides belonging to the non-canonical renin-angiotensin system, such as angiotensin-(1–7), angiotensin-(1–9), AT2R and Mas receptors, and the enzymes that participate in those reactions, remain to be determined.

3. **Role of oxidative stress in essential hypertension**. It is of interest to note that myofibrils contraction will occur whenever ROS increases within the vascular smooth muscle cells, including the above-mentioned receptor pathway, but also any other ROS source. The best characterized ROS source in the vascular wall is NADPH oxidase, but several enzymes may also contribute in this process (xanthine oxidase, uncoupled eNOS, iNOS, and mitochondrion, among others). Together with the direct ROS-induced vasoconstriction, the reduction in NO bioavailability and downregulation of prostacyclin synthase are mechanisms able to account for the production of imbalance between vasodilators and vasoconstrictors in favor of the latter.

4. **Oxidative stress and secondary hypertension**. Although most cases of hypertension are essential, identifiable causes account for 5%–10% of cases. The various etiologies include renal and endocrine origin. The mechanisms include the contribution of the vasomotor areas of the central nervous system and the renin-angiotensin-aldosterone axis, among others, with oxidative stress involved in all of them. Increased reactive oxygen species is associated to a wide spectrum of effects on the vascular wall, ranging from endothelial dysfunction, to intima-media thickness, and other vascular events leading to increased blood pressure.

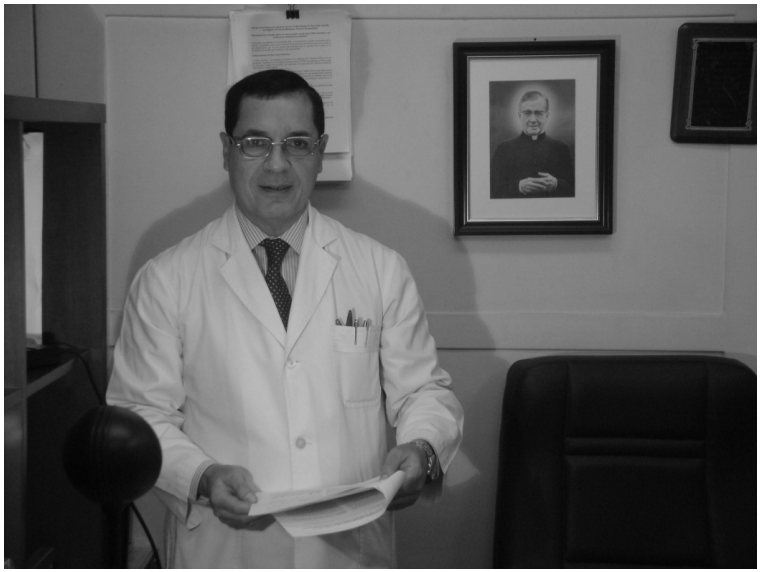
5. **Antioxidants and therapy of hypertension**: l**ooking forward.** The relationship between oxidative stress and blood pressure modulation results from the vascular vasoconstrictor response to reactive oxygen species, but also to a decreased bioavailability of vasodilator mediators. Therefore, from a therapeutic point of view of hypertension, it is relevant to counteract the occurrence of oxidative stress. Indeed, most of antihypertensive drugs currently in use have antioxidant

properties. In addition, it is of interest that refractory hypertension has been increasing despite new drugs added to the treatment of patients. Other exogenous naturally occurring antioxidants, such as antioxidant vitamins or polyphenols, need to be studied in clinical trials to explore the possibility of reducing refractory hypertension in potentiating the antioxidant defense system with safe and easily available compounds.

The aim of this book is to provide updated research advances consistent with the association of oxidative stress and human hypertension, presenting a therapeutic target of a relevant health problem with a higher mortality despite the use of a great deal of resources. Particularly notorious is the need to use three or more antihypertensive drugs against resistant hypertension. However, further research of antioxidants as potential adjunct antihypertensive agents is still needed.

About the Editor

Dr. Ramón Rodrigo, Master's in Medical Sciences, is Full Professor at the Faculty of Medicine, University of Chile, Institute of Biomedical Sciences, Molecular and Clinical Pharmacology Program. As a researcher in the field of oxidative stress he has published five books as editor, 40 book chapters, and about 250 articles in journals, having more than 8500 citations. As an academic of medical sciences, he is a member of the Board of two Doctorate and one Master Programs of the University of Chile, he has been coeditor of a textbook, and directed numerous Master and Doctorate theses.



CHAPTER 1

ENDOTHELIUM AND VASCULAR HOMEOSTASIS

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Abstract

Endothelial function, its interactions, and its alteration and malfunction, are responsible for the development of several vascular diseases. The NO pathway, endothelium-derived hyperpolarization, and several biological signals such as angiotensin II, acetylcholine, endothelins, or eicosanoids are part of the many endothelial-dependent processes in arterial function. Reactive oxygen species (ROS) are a family of highly reactive molecules which play a crucial role in a large number of biological processes and systems. ROS react with several key components to vascular function. While many of these interactions are well understood and documented, such as the NO pathway, other processes have been more difficult to develop into a coherent narrative regarding their effects, such as ROS interactions with endothelium-derived hyperpolarization. This chapter aims to develop a

comprehensive overview on how oxidative stress and reactive oxygen species interact with the endothelium and vascular homeostasis, becoming a key pathophysiological component in the development and sustainment of hypertension.

Abbreviations

ACh	Acetylcholine
ADMA	Asymmetric dimethylarginine
Ang II	Angiotensin II
BH2	Dihydropterin
BH4	Tetrahydrobiopterin
Cav-1	Caveolin-1
COX	Cyclooxygenase
ECM	Extracellular matrix
EDHF	Endothelium-derived hyperpolarizing factor
eNOS	Endothelial isoform of nitric oxide synthase
EP	Prostaglandin E2 receptor
ET-1	Endothelin 1
ETA	Endothelin-1 A receptors
ETB	Endothelin-1 B receptors
H2O2	Hydrogen peroxide
iNOS	Inducible nitric oxide synthase
IL-1B	Interleukin-1β
MAPK	Mitogen activated protein kinase
MMP	Matrix metalloproteinases
mPGES-1	Microsomal prostaglandin E synthase-1
NADPH	Reduced nicotinamide adenine dinucleotide
NE	Norepinephrine
NO	Nitric oxide
NOS	Nitric oxide synthase
Nox	NADPH oxidases
O2•−	Superoxide anion
ONOO-	Peroxynitrite
PGE2	Prostaglandin E2
PGH	Prostaglandin H
PGI2	Prostaglandin I2 (Prostacyclin)
ROS	Reactive oxygen species
SNA	Sympathetic nervous system activity
TRP	Transient Receptor Potential
TRPV4	TRP vanilloid type 4

TxA2	Thromboxane A2
VSMC	Vascular smooth muscle cells
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase

1. Vascular structure and determinants of blood pressure

Blood pressure is determined as the product of cardiac output and vascular resistance. When it comes to primary hypertension, the main pathophysiological mechanism involved in increased blood pressure is an increase in vascular resistance. According to the Poiseuille equation, the resistance to blood flow is inversely proportional to the fourth power of the vessel radius. Thus, a decrease in the lumen diameter of an artery increases the resistance proportionally more, raising arterial blood pressure. Therefore, changes in the luminal diameter of vessels are the main determinants of blood pressure.

Vascular tone and wall thickness are the main determinants of arterial luminal diameter. A brief description of each component of the vascular structure is discussed below.

1.1 Endothelium

The endothelium is a single layer of cells located at the intima, the innermost layer of a vessel. It is a complete organ which participates in the vascular system regulation. The endothelium controls vascular function in response to multiple stimuli, including hormones, neurotransmitters, and vasoactive inputs. The vascular regulation is performed through various vasoactive factors, which primarily control the muscle layer of the vessel. Its function is not limited to the vascular tone though—it also has multiple roles in the organism having an effect on platelet aggregation, inflammation, and coagulation homeostasis.

The endothelium-derived factors that participate in vascular regulation can be either vasodilators or vasoconstrictors. Among vasodilators we can find nitric oxide (NO), prostaglandin I2 (PGI2, prostacyclin), and endothelium-derived hyperpolarizing factor (EDHF). On the other hand, vasoconstrictive factors include thromboxane A2 (TxA2) and endothelin 1 (ET-1). Disturbances in these factors can lead to an increase in vasoconstriction, due to either reduced availability of vasodilators, or to an excess of vasoconstrictors, leading to pathologies such as arterial hypertension.

1.2 Smooth muscle cells

As previously mentioned, the main determinants of blood pressure are vascular tone and wall thickness. Both mechanisms depend on the vascular smooth muscle cells (VSMC) to produce an effect. VSMC can modify the luminal diameter through short-term regulation of the vascular tone, mainly determined by endothelial activity. VSMC also regulate wall thickness, which directly affects the luminal diameter. This can produce long-lasting effects in blood pressure. The structural remodeling happening in the layer of VSMC is determined by a plethora of cell signals, some being endothelium-mediated, cardiovascular hormones-mediated, or physical stimuli. There are several cell pathways involved in proliferation and hypertrophy, but Ras/mitogen activated protein kinase (MAPK) pathway signaling has been demonstrated to be an important pathway for this process. Oxidative stress has been demonstrated to cause cell proliferation and muscle layer hypertrophy, mainly through activation of the MAPK pathway. It is currently being discussed which receptors and activators of the MAPK pathway are the ones involved. It has been proposed that reactive oxygen species interact with proto-oncogene tyrosine-protein kinase Src (c-Src), a protein from tyrosine kinase receptors, which may change the reactivity of the receptors to different signals [1]. Some of these include the insulin-like growth factor receptor, the epidermal growth factor receptor, and the platelet-derived growth factor receptor. MAPK signaling contributes to enhanced cell survival signals, cell division, and expression of Gqα/PLCβ1 proteins, resulting in VSMC hypertrophy [2].

1.3 Adventitia

The adventitia layer of the vascular wall is a connective tissue layer mainly composed of fibroblasts and collagen. Most of the adventitia is extracellular matrix, and the main protein in the extracellular matrix is collagen. Changes in adventitia thickness and composition lead to changes in vascular homeostasis, mainly elasticity, which has been shown to decrease in arterial walls of the elderly.

Increased reactive oxygen species production by adventitial fibroblast NADPH oxidase (Nox) has been shown to cause vascular remodeling, increasing wall thickness, and reducing vascular elasticity. Nonetheless, it has been proposed that the importance of adventitia in vascular homeostasis is contributing to oxidative stress in the vessel wall, distributing ROS to other components of the arteries. This has been demonstrated by Ang II stimulation. Additionally, Ang II in the adventitia causes the release of

vasoactive hormones such as growth factors and ET-1, which may further regulate vascular structure and function via autocrine or paracrine signaling mechanisms [3].

2. Endothelium-dependent vasoregulators

2.1 Nitric oxide

In 1984, Furchgott and Zawadzki described that the endothelium-dependent vasodilation was preceded by an increase in cyclic guanosine monophosphate (cGMP), proposing an endothelium-derived relaxing factor as responsible for this increase. That factor is in fact, nitric oxide [4].

Nitric oxide can cause vasodilation either by soluble guanylyl cyclase in a dependent or independent manner. Within the VSMC, NO binds and activates the soluble guanylyl cyclase enzyme, which converts guanosine triphosphate into cyclic guanosine monophosphate. The latter activates cGMP-dependent protein kinase, leading to lower cytosolic Ca^{2+} concentrations and subsequent vasodilation. NO also stimulates the endoplasmic reticulum calcium ATPase, which also reduces the intracellular Ca^{2+} concentration and produces vasodilation. NO is known to have pleiotropic effects besides the aforementioned vasodilation. Among them we find a decrease in inflammation, vascular cell proliferation, platelet adhesion, and tissue factor inhibition. NO is mainly produced by the endothelial isoform (eNOS) of nitric oxide synthase (NOS). There are other isoforms, such as neuronal and inducible NOS. In hypertension, there is an alteration in the expression of these enzymes, but also a compensatory upregulation of neuronal isoform [5, 6].

2.1.1 Nitric oxide synthase reaction

NOS catalyzes the reaction of molecular oxygen with the amino acid substrate L-arginine to produce L-citrulline and NO [7]. L-arginine transport is impaired both in hypertensive and normotensive subjects with a genetic background of essential hypertension [8]. Furthermore, L-arginine supplementation improves endothelial dysfunction in hypertension [9].

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS [10, 11] and also inhibits cationic amino acid transporters that supply intracellular NOS with L-arginine from the plasma. ADMA can be increased by reduced nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) activity [12] and oxidative stress [13, 14]. Oxidative stress-dependent increases in circulating ADMA could lead to

eNOS uncoupling [15], vasoconstriction [16], and a subsequent marked increase in blood pressure [15, 17, 18]. In fact, patients with hypertension show significantly higher plasma concentrations of ADMA and a reduced plasma ratio of L-arginine/ADMA [19].

Tetrahydrobiopterin (BH4) is an essential cofactor for the catalytic activity of all three NOS isoforms, as it increases L-arginine binding and stabilizes the active dimeric form of the enzyme [20–22]. Dihydrofolate reductase catalyzes the regeneration of BH4 from its oxidized form, dihydropterin (BH2) [23, 24]. Dihydrofolate reductase expression can be downregulated by endothelial NADPH oxidase-derived H_2O_2, resulting in BH4 deficiency and thus uncoupling of eNOS [25]. Uncoupled eNOS produces superoxide, leading to the oxidation of BH4 to BH2, which leads to further eNOS uncoupling, resulting in a positive feedback mechanism that perpetuates oxidative stress [19].

2.1.2 Nitric oxide synthase interaction with caveolae, caveolins, and oxidative stress

Under basal conditions, eNOS is inactive, due to its union with membrane invaginations called caveolae [26]. Oxidized low-density lipoproteins (LDL), regarded as a representative parameter of oxidative stress, causes depletion of caveolae cholesterol in cultured endothelium via the scavenger receptor CD36, leading to eNOS redistribution away from the plasma membrane and diminished capacity to activate the enzyme [27]. Due to the provision of cholesterol esters by high-density lipoproteins (HDL), this molecule maintains caveolae cholesterol content, retains eNOS in the domain, and thereby preserves the capacity of eNOS activation [28].

Caveolins are the main coat proteins of caveolae. Studies in endothelial cells demonstrate that eNOS has the capacity to directly interact with caveolin-1 (Cav-1) or caveolin-3 and that this interaction results in inhibition of NO production [29]. Furthermore, in vivo experiments support the role of caveolin-1 (Cav-1) as a negative regulator of eNOS [30]. In fact, Cav-1 deletion prevents hypertensive vascular remodeling induced by angiotensin II [31], while the presence of caveolae with Cav-1 expression increased significantly in the aortas of rats with pulmonary hypertension [32]. It has been shown that Interleukin-1β (IL-1B) induces the upregulation of Cav-1 [33], while NADPH oxidase-derived ROS are involved in human neutrophil IL-1β secretion [34]. Thus, we propose that oxidative stress-dependent production of IL-1β could be involved in Cav-1 induced hypertension.

2.2 Norepinephrine

To this date, several studies assessing either indirect or direct markers of sympathetic function have provided compelling evidence that in early stages of hypertension or in young hypertensive subjects, the sympathetic nervous system is upregulated. Norepinephrine (NE) is a powerful vasoconstrictor agent released by the sympathetic nervous system on adrenergic receptors of the vascular smooth muscle. The activation of α1-adrenergic receptors by NE can lead to VSMC proliferation via the MAPK pathway. On the other hand, oxidative stress can mediate a central activation of the sympathetic nervous system by overexpression of iNOS, resulting in increased blood pressure [35].

2.3 Angiotensin II

Angiotensin II (Ang II) is a potent vasoactive peptide that can be formed in several vascular beds, as long as they contain angiotensin I converting enzyme. When Ang II production increases above normal levels, it can lead to vascular remodeling and endothelial dysfunction in association with increased blood pressure levels. Also, despite the commonly described context of enhanced sympathetic nervous system activation in hypertension, it has been described that chronically elevated plasma levels of Ang II lead to a salt-sensitive form of hypertension that is associated with a differential pattern of peripheral sympathetic outflow. This phenomenon is called the "Ang II-salt sympathetic signature," being characterized by a transient reduction in sympathetic nervous system activity (SNA) to the kidneys, no change in SNA to skeletal muscle, and a delayed activation of SNA to the splanchnic circulation. Thus, SNA is differentially regulated in Ang II-salt rats, the splanchnic vascular bed being the primary target of the sympathetic nervous system in this model of hypertension. [36].

On the other hand, in terms of a possible relationship between oxidative stress and Ang II dependent hypertension, a wealth of evidence has emerged implicating Ang II-induced ROS generation in the pathogenesis of hypertension, NADPH oxidase being possibly the predominant source of derived ROS production in the brain [37]. Also, reactive oxygen species may play a role by impairing sympathetic vasoregulation in the skeletal muscle in the context of this type of hypertension. In fact, chronically elevated Ang II increases muscle ROS, which disrupts the normal NO-dependent attenuation of sympathetic vasoconstriction [38]. On the other hand, prostaglandin E2 (PGE2) acting on endothelin-1 receptors contributes

to excessive ROS levels derived from NADPH oxidase and mitochondria in response to Ang II [39, 40].

2.4 Acetylcholine

Acetylcholine (ACh) is an endothelium-dependent vasodilator released after its generation following mitochondrial production of acetyl-CoA. Once liberated, ACh triggers calcium release from the internal store of endothelial cells, nitric oxide production, and thus, artery relaxation. The nitric oxide depletion caused by ROS, discussed before, worsens the ACh-mediated vasodilation response [41]. To this date, it has been widely described that endothelium-dependent vasodilation to ACh is reduced in the forearm of essential hypertensive patients [42]. Thus, it is fair to hypothesize that the increase of availability of ACh, resulting from the effect of antiacetylcholinesterases, may prevent autonomic imbalance and reduce inflammation, yielding beneficial effects for cardiovascular disorders in hypertension, which is exactly what happens with the administration of Donepezil in spontaneously hypertensive rats [43].

2.5 Endothelium-derived hyperpolarizing factor

In addition to other vasodilating factors, endothelial cells relax the vascular muscle layer through hyperpolarization of the smooth muscle cells. The mechanism through which the endothelium produces hyperpolarization in the smooth muscle cells varies between vascular types and species, but there are two well-documented mechanisms: diffusible factors and contact-mediated pathways. It seems that in normal conditions, the endothelium-derived hyperpolarizing factor (EDHF) system serves as a vasodilator reserve. In the case of NO pathway dysfunction or downregulation, upregulation of EDHF has been observed. The interplay between NO and EDHF is complex and not properly understood. It is estimated that, in normal conditions, the NO pathway inhibits EDHF activity through various mechanisms, such as inhibition of cytochrome P-450 enzyme activity [44], inhibitory effect on cation channels [45], and reduced permeability of gap junctions [45, 46].

Hydrogen peroxide (H_2O_2) is known to induce vascular relaxation through EDHF activation [47, 48]. Therefore, H_2O_2 behaves both as an inhibitor of the NO vasodilator system, and as a vasodilator through the EDHF system. A reduction of EDHF function has been seen in prolonged hypertension, mainly through endothelial dysfunction [49]. The precise mechanisms in which this takes place have not been fully described.

Myoendothelial gap junctions are responsible for the electrical propagation of hyperpolarization from endothelial cells to smooth muscle cells, but it seems to have little impact in the reduction of EDHF function [50].

2.5.1 Endothelium-derived hyperpolarizing factor and oxidative stress

As discussed before, EDHF's activity is probably impaired in hypertension, although there are studies which argue that EDHF function is the same in normotensive and hypertensive subjects, and mainly acts as a backup vasodilator to compensate for decreased NO pathway function. We think this might be the case in physiological conditions and early stages of hypertension pathophysiological development. This has been observed by an initial upregulation of EDHF system activity in early hypertension, and then impairment in later stages of the disease [51].

2.5.2 Endothelium-derived hyperpolarizing factor and hypertension

Vascular cells produce a number of different ion channels (SKCa, IKCa, KIR, KATP, Kv, TRPs, CaCCs), and alterations in their expression have been documented in hypertension [52]. A study argues that the impaired EDHF function observed in animal models of hypertension is caused by alterations of function and expression of the aforementioned channels, thus suggesting that the alterations in ion channels could become therapeutic targets for the prevention and reversal of EDHF dysfunction and endothelial dysfunction that comes with hypertension [50].

2.5.3 Transient receptor potential

Transient receptor potential (TRP) channels are non-selective cation channels, and they play a role in hyperpolarization generation in the endothelium, regulating vascular function [53–55]. Specifically, TRP vanilloid type 4 (TRPV4) may be involved in this function [55, 56]. TRPV4 is downregulated in spontaneously hypertensive rats, and it seems to be a consequence of hypertension, due to the preserved number and function that prehypertensive patients show [56]. Also, TRPV4 knockout mice presented a decrease in EDHF function [57]. Some TRP channels seem to be regulated through direct binding of ROS. Regulation of TRPM2 is distinctive and involves direct binding of oxidant-derived second messengers [58]; however, its role in EDHF function is unclear.

2.6 Endothelin 1

Endothelin 1 (ET-1) is widely known for being a potent vasoconstrictor peptide. Its continuous release from endothelial cells can be inhibited by NO [59, 60]. In return, ET-1 has a strong inhibitory effect on NO-mediated vasodilation [61], but it also reduces β-adrenergic receptor-dependent relaxation [62], being a potent antivasodilatory factor. At elevated concentrations, ET-1 can promote inflammation and vascular smooth muscle cell proliferation [63, 64].

ET-1 effects depend on the activation of ETA and ETB receptors [65, 66]. ETB participates in ET-1 clearance and in the release of endothelial prostacyclin and NO with subsequent vasodilation. ETB inhibition increases circulating ET-1 levels and blood pressure in healthy subjects. In contrast, inhibition of ETA receptors causes coronary dilation, increased coronary blood flow, and decreased coronary resistance [66, 67]. The production of ET-1 is regulated at a genetic level [68], being upregulated by inflammatory factors (such as transforming growth factor beta, tumor necrosis factor alpha, interleukins, insulin, and Ang II), and downregulated by NO, PGI2, hypoxia, and shear stress [68–70].

We can find an example of ET-1 upregulation in the vascular wall of salt-dependent models of hypertension [71, 72]. Furthermore, an increase of ET-1 plasma levels is also induced by acute mental and physical stress in human adults and adolescents, which correlates with stress-induced increases in blood pressure in prehypertensive young adults with verified family histories of cardiovascular disease, being the stress-induced release of ET-1 probably involved in the acute stress-induced pressor response [73, 74]. Reactive oxygen species contribute to this ET-1 induced pressor response to acute stress. In fact, the increase in reactive oxygen species occurs downstream of ET-1 receptor activation [75]. Also, the increased vascular oxidative stress in animal models of hypertension is associated with activation of the ET (endothelin) system via ET receptors [76], this oxidative stress being independent of NADPH oxidase and probably mediated by the mitochondria [77]. Nevertheless, it has been shown that ET-1 augments vascular superoxide production at least in part via an ET(A)/NADPH oxidase pathway in low-renin mineralocorticoid hypertension [71].

2.7 Eicosanoids

Prostacyclin (PGI2) and TxA2 are eicosanoids produced by oxidation of arachidonic acid catalyzed by cyclooxygenase (COX) enzymes [78]. These

compounds have opposite effects on vascular tone, with prostacyclin being responsible for vasodilation, while TxA2 produces vasoconstriction. Thus, the balance between the two contributes to the homeostatic regulation of normal blood pressure by the subsequent modification of the vascular resistance [79]. The disturbance of this balance can lead to hypertension, with increased levels of TxA2 and at the same time, reduced levels of prostacyclin. In fact, prostacyclin knockout mice present increased rates of hypertension, increased fibrosis, vascular injury, and kidney infarction [80], while transgenic overexpressing PGIs mice are protected against hypoxic pulmonary hypertension [81]. Also, elevations in blood pressure and cardiac hypertrophy of hypertensive rat models can be attenuated when the TxA2 receptor is knocked out [82]. Now, in the clinical context, hypertensive men exhibit an increased TxA2 to prostacyclin ratio when compared to normotensive subjects. Impaired prostacyclin biosynthesis in hypertensive patients could account for the typical increased vascular resistance and complications of the hypertensive state [83].

The TxA2 to prostacyclin ratio is also increased during preeclampsia, being partially responsible for hypertension, increased vascular reactivity, and increased platelet aggregation associated with the disease [84]. Oxidative stress is considered a major molecular determinant of preeclampsia [85]. There is, in fact, a relationship between oxidative stress and increased TxA2 in preeclampsia. Glutathione peroxidase—an enzyme that converts lipid hydroperoxides to less reactive alcohols—may be deficient in this disease. Consequently, lipid hydroperoxides result increased, thus inhibiting PGI2 synthase enzyme activity while prostaglandin H synthase, the cyclooxygenase component is stimulated. Therefore, increased TxA2 to prostacyclin ratio accounts for vasospasm and exacerbation of placental ischemia, increased cell damage, and increased lipid peroxidation, enhancing the oxidative stress. Patients with this disease show higher total plasma vitamin E levels [86] when compared to normotensive pregnancies, possibly reflecting enhanced antioxidant defense in response to oxidative stress in women with preeclampsia.

During inflammatory conditions, the production of eicosanoids—particularly PGE2—is enhanced by the action of the inducible COX-2. Microsomal prostaglandin E synthase-1 (mPGES-1), a COX-2 downstream enzyme, controls both baseline and inducible PGE2 production [87]. PGE2 modulates vascular tone through four PGE2 receptor (EP) subtypes (EP1–4). EP1 and EP3 receptors mediate excitatory and contractile effects, whereas EP2 and EP4 receptors mediate inhibitory and vasodilator effects [40]. Increased PGE2 production and microsomal prostaglandin E synthase-1 (mPGES-1) expression are observed in vessels from several models of

hypertension and in peripheral cells from hypertensive patients. Furthermore, mPGES-1-derived PGE2 acting on EP1/EP3 receptors and activating JNK/ERK1/2 pathways contributes to the excessive ROS levels derived from NADPH oxidase and mitochondria in response to Ang II [39, 40]. A summary of all these factors is presented in Table 1-1.

3. Role of reactive oxygen species in vascular homeostasis

The situation most frequently discussed when talking about reactive oxygen species and the endothelium is endothelial dysfunction through oxidative stress, meaning the loss of regulation of ROS mechanisms. Nevertheless, it is widely known that physiological ROS production plays important roles in various cellular processes, such as gene expression, proliferation, and in the case of the endothelium, even vasodilation through EDHF system activation [47, 48, 88].

The loss of regulation of ROS systems, also known as oxidative stress, causes pathophysiological phenomena including ischemia reperfusion damage, atherogenesis, and chronic inflammation, among many others. Specifically, it generates endothelial dysfunction [88, 89].

This is evident in hypertension, as there is an established impairment of antioxidant activity. Antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) are significantly decreased in prehypertension and in stage I and stage II hypertension groups when compared to control individuals [90].

3.1 Reactive oxygen species sources in the vasculature and their interactions

3.1.1 NADPH oxidases

NADPH oxidases (Nox) are the major ROS source in the endothelium [91]. They can be upregulated by various signals, such as Ang II, vascular endothelial growth factor, and platelet-derived growth factor, among others [92]. Endothelial-specific Nox-2 overexpression in mice increases Ang II-induced hypertension, vascular oxidative stress, endothelial dysfunction, vascular fibrosis, and left ventricular diastolic dysfunction [93, 94]. It can be said that in pathophysiological conditions, Nox activity translates into worse hypertension outcomes, and it has been suggested to become a pharmacological target [91]. But there are also a number of studies pointing to better endothelial function and outcomes when Nox activity is increased in the endothelium. Overexpression of Nox4 produces enhanced endothelium-

derived hyperpolarization, but not altered nitric oxide bioactivity, and decreased blood pressure compared to wild mice [95]. It is worth noting that Nox4-overexpression mice only had this alteration and did not have hypertension. It was suggested that perhaps Nox4 was “the good Nox” homologue [96]. Proinflammatory mediators that induce Nox1 or Nox2 appear to downregulate Nox4 expression [97]. Nox4 is the only vascular Nox homologue that directly produces hydrogen peroxide (H2O2), unlike the other homologues that primarily produce superoxide anion (O_2•−), and thus do not scavenge NO nor produce peroxynitrite (ONOO-) [98]. It was recently concluded that Nox4 protects from chronic hemodynamic overload-induced cardiac remodeling, by demonstrating that Nox4 knockout mice had worse pressure overload-induced cardiac remodeling and dysfunction when compared to wild-type mice [99]. However, the available data do not allow concluding that Nox4 upregulated expression can lead to better hypertension control, since most of the studies were not performed using a hypertension animal model. Therefore, it could be said that Nox4 indeed plays an important role in preserving normal endothelial function, but its role in oxidative stress conditions or once endothelial dysfunction is already established remains unclear.

Nox5 is the latest identified NADPH oxidase, and unlike the other subtypes, it does not require any NADPH oxidase subunit to function, being also capable of producing ROS at lower Ca2+ levels. Another feature that makes Nox5 different is that it is not present in mice like the other Noxes, so it is especially hard to experiment with. In vascular cells, Nox5 isoforms Nox5α and Nox5β are its major ROS sources, being activated by thrombin, platelet-derived growth factor, Ang II, and ET-1.

Nox5 has been implicated in cell proliferation, angiogenesis, and migration. In animal models, Nox5-derived ROS participate in VSMC proliferation and migration in atherogenesis. Moreover, Nox5 expression is increased in several cardiovascular diseases, among which we find hypertension. In fact, Nox5 expression in mice is associated with renal function impairment and higher blood pressure [100].

3.1.2 Xanthine oxidase

Xanthine oxidase (XO) is a hepatic and vascular endothelium enzyme that catalyzes the oxidation of hypoxanthine and xanthine to form superoxide (•O_2−), leading also to the production of uric acid, NO, and ROS, thus having a relevant role in oxidative stress. Furthermore, this enzyme is also upregulated by NADPH oxidase activation, being a possible mechanism of an oxidative stress vicious circle [101]. Xanthine oxidase

exists in two different forms: xanthine dehydrogenase (XDH) and XO. The cellular increase of the XO to XDH ratio would be a critical step in some point of the development of endothelial dysfunction and hypertension, among other cardiovascular diseases. In fact, the enzyme's activity is increased both in hypertensive patients and in patients with Ang II-associated coronary disease, while spontaneously hypertensive rats show higher levels of the endothelial form of XO and increased ROS production, leading to increased vasoconstriction [102, 103].

It has been demonstrated that the enzyme inhibitor allopurinol can improve cardiac hypertrophy in spontaneously hypertensive rats but has a minimal impact on blood pressure. Thus, in terms of the specific role of this enzyme in the pathogenesis of hypertension, XO would be participating in the end organ damage rather than in the development of the disease itself [104]. For example, both endothelial XO and plasma XO activity are increased in human atherosclerotic plaques, suggesting that XO-derived superoxide contributes to the development of hypertension-induced atherosclerosis [105–107]. Therefore, plasmatic uric acid has been proposed as a potential oxidative stress biomarker [107, 108]. On the other hand, while experimental models of hypertension exhibit increased XO activity in the kidney, long-term inhibition of XO with allopurinol reduced renal XO activity without lowering blood pressure, supporting again that XO would not necessary participate in the development of the disease itself but in the end organ damage produced by hypertension [109].

3.2 Reactive oxygen species and notch signaling

In the endothelium, notch signaling regulates endothelial functions through influence in other pathways responsible for angiogenesis, inflammation, and apoptosis. The notch pathway has been associated with endothelial dysfunction [110].

ROS have demonstrated to regulate angiogenesis through notch signaling; even noting notch effects on cell proliferation, migration, and adhesion are mediated by ROS, and Nox4-dependent phosphorylation of vascular endothelial growth factor receptor [111, 112]. Moreover, the notch pathway controls Nox4 activity and ROS production [111].

4. Chronic vascular remodeling

The process of chronic vascular remodeling in hypertension requires the phenotypic change of VSMC from a contractile to a proliferative and synthetic phenotype, thus leading to vascular hypertrophy and with that, to