



Review

Endothelial and vascular smooth muscle dysfunction in hypertension

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ABSTRACT

The development of essential hypertension involves several factors. Vascular dysfunction, characterized by endothelial dysfunction, low-grade inflammation and structural remodeling, plays an important role in the initiation and maintenance of essential hypertension. Although the mechanistic pathways by which essential hypertension develops are poorly understood, several pharmacological classes available on the clinical settings improve blood pressure by interfering in the cardiac output and/or vascular function. This review is divided in two major sections. The first section depicts the major molecular pathways as renin angiotensin aldosterone system (RAAS), endothelin, nitric oxide signalling pathway and oxidative stress in the development of vascular dysfunction. The second section describes the role of some pharmacological classes such as i) RAAS inhibitors, ii) dual angiotensin receptor-neprilysin inhibitors, iii) endothelin-1 receptor antagonists, iv) soluble guanylate cyclase modulators, v) phosphodiesterase type 5 inhibitors and vi) sodium-glucose cotransporter 2 inhibitors in the context of hypertension. Some classes are already approved in the treatment of hypertension, but others are not yet approved. However, due to their potential benefits these classes were included.

1. Introduction

The development of arterial hypertension can be influenced by anatomical, genetic, endocrine, humoral, hemodynamic, environmental and neural factor. However, the mechanistic pathways by which essential hypertension develops have not yet been fully understood. Vascular dysfunction, characterized by endothelial dysfunction (ED), chronic low-grade inflammation and structural remodeling, plays an important role in cardiovascular diseases including the initiation and maintenance of arterial hypertension [1–7].

In the healthy vessel, the endothelium senses blood flow and participates in a variety of physiological function including i) the release of

contractile and relaxing substances that control the vascular smooth muscle tone ii) the release of growth factors aiming at accelerating reendothelialization (e.g. VEGF and PDGF); iii) the secretion and expression of prothrombotic (tissue factor, plasminogen activator inhibitor 1, P-selectin, E-selectin, vascular cell adhesion protein 1) and antithrombotic (e.g. thrombomodulin, heparan sulphate, tissue factor pathway inhibitor, tissue plasminogen activator, ectonucleoside triphosphate diphosphohydrolase-1) factors to promote and prevent, respectively, clot formation; and iv) anti-inflammatory (e.g. IL-4, IL-10, TGF- β) and pro-inflammatory mediators (e.g. TNF α , IL-6, MCP-1, NLRP3 inflammasome pathway) [4,8–14]. The imbalance between the production of vasodilating/vasoconstricting, anti-/proinflammatory and

Abbreviations: ACE, angiotensin converting enzyme; ACE-2, angiotensin converting enzyme 2; ADH, antidiuretic hormone; ADMA, asymmetric dimethylarginine; AGT, angiotensinogen; ALDO, aldosterone; ANG I, angiotensin I; ANG II, angiotensin II; ANP, atrial natriuretic peptide; ARNI, dual angiotensin receptor-neprilysin inhibitors; AVP, arginine vasopressin; BH4, tetrahydrobiopterin; BNP, brain natriuretic peptide; cGMP, cyclic 3'-5' guanosine monophosphate; COX, cyclooxygenase; EC, endothelial cells; ED, endothelial dysfunction; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; ET, endothelin; FMD, flow mediated dilation; IL, interleukin; JNK, c-Jun N-terminal kinase; KO, knockout; MAPK, p38 mitogen-activated protein kinase; MAPKs, mitogen-activated protein kinases; MCP-1, Monocyte chemoattractant protein-1; MR, mineralocorticoid receptor; NEP, neprilysin; NET, neutrophil extracellular traps; NHE-1, sodium-hydrogen antiporter 1; NO, nitric oxide; NOS, nitric oxide synthases; NOX, NADPH Oxidases; NLRP3, NLR family pyrin domain containing 3; NT-proBNP, N-terminal fragment of BNP; PDGF, platelet derived growth factor; RAAS, Renin Angiotensin Aldosterone System; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; SGLT2, sodium-glucose cotransporter-2; SHR, Spontaneously Hypertensive Rats; SMC, smooth muscle cell; TGF- β , transforming growth factor β 1; TNF- α , tumoral necrosis factor α ; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells.

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anti-/pro-atherothrombotic mediators leads to ED (Fig. 1 a, b). The reactive oxygen species (ROS), when produced in large amount in the vascular and endothelium layers, promote ED, vascular smooth muscle hypercontractility, inflammation, lipid peroxidation and thickening of the vessel wall [15].

Impairment of endothelium-dependent vasodilation has been largely documented in hypertension. The Framingham Heart Study [16] was one of the first population-based studies showing that systolic blood pressure was inversely correlated with flow-mediated dilation (FMD), which is a largely accepted, accurate and noninvasive method to assess endothelial function to date. However, its use in daily clinical practice is not recommended yet by clinical guidelines for assessing vascular dysfunction.

The present review is focused on i) the major pathways involved in the impairment of vascular function and ii) experimental and, when appropriate, clinical evidences about the role of some pharmacological classes in improving vascular function in hypertension. Some classes are already approved on clinical settings to treat patients with arterial hypertension. Others classes were not yet approved but were included due to their potential use in hypertension.

2. Major molecular pathways involved in the vascular dysfunction

2.1. Role of renin angiotensin aldosterone system (RAAS)

The Renin Angiotensin Aldosterone System (RAAS) plays a fundamental role in the control of blood pressure, body salt and fluid homeostasis [17,18]. The classical components of RAAS includes renin, angiotensinogen (AGT), angiotensin I (ANG I), angiotensin converting enzyme (ACE), aldosterone (ALDO) and its most potent effector angiotensin II (ANG II) (Fig. 2a). A counter-regulatory pathway includes angiotensin 1-7 (ANG 1-7)-Mas receptor (MasR) axis and the angiotensin converting enzyme 2 (ACE-2), which converts ANG II to Ang 1-7 (Fig. 2a). Local RAAS also exists in specific tissues such as brain, heart, vascular and perivascular adipose tissues, exerting paracrine and autocrine effects, independently of the systemic RAAS regulation, and

playing a critical role in the pathogenesis of a variety of cardiovascular diseases [19,20]. In fact, with the exception of renin, all components of the RAAS are produced in the vasculature [21,21]. Additionally, alternative pathways to increase plasmatic ANG II generation by chymases is described in rodent and human cardiovascular tissues [23–25]. Experimental studies showed that chymases inhibition significantly reduces ANG II plasma and blood pressure in spontaneously hypertensive rats (SHR; 16-week-old; [24]) and ANGII increased renal production and hypertension in ANG I-infused ($1 \mu\text{g/Kg}^{-1}$, 28 days) high-salt diet treated mice (4% NaCl; [26]), suggesting another promising approach for the treatment of hypertensive patients. Furthermore, neprilysin (NEP), a metalloproteinase with broad substrate specificity and physiological functions, effectively cleaves ANG I and ANG II to generate ANG 1–7 to a greater extent than ACE-2, thus modulating the RAAS axis [27] (Fig. 2a).

A common approach to directly assess RAAS contribution in hypertension are ANG II-dependent models. Although the animals uniformly exhibit hypertension, it is important to acknowledge several limitations in this model, since pharmacological doses of ANG II (frequently $\geq 400 \text{ ng/Kg}^{-1}/\text{min}^{-1}$) may not mimic the pathophysiology in humans [28]. Similarly, care should be taken in analyzing cell culture studies as they may employ high concentrations of ANG II, often in the nanomolar range, while the concentration of ANG II in human plasma is about 20 pM in healthy subjects [29].

Both local and circulating ANG II activate the classical angiotensin receptors AT_1R and AT_2R , localized in the rat, mouse, monkeys and human kidney [30–32] and in several cell types, including endothelial and vascular smooth muscle cells [22]. The majority of ANG II effects, physiological or deleterious, are mediated by AT_1R activation [33,34], while activation of AT_2R , although not fully understood, may counterbalance the effects AT_1R activation, promoting moderate vasodilation, natriuresis, anti-angiogenesis, anti-proliferation and anti-fibrotic responses in several tissues including endothelium, vascular smooth muscle, heart, brain, and kidney [35–39] (Fig. 2).

An important mechanism by which ANG II induces vascular remodeling is through the activation of mitogenic factors, including the epidermal growth factor receptor (EGFR) [40]. The stimulation of EGFR leads to the activation of Src family kinases promoting vascular smooth muscle cells (VSMCs) proliferation, in addition to Ca^{2+} channels activation, enhancing vascular myogenic reactivity [41]. Furthermore, deletion of EGFR prevented ANG II infusion ($1000 \text{ ng/Kg BW/min}$, 3 weeks)-induced VSMCs hyperplasia and hypertrophy in mice [42]. Mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase (ERK) 1/2, p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK) are also critical mediators of ANG II induced VSMCs phenotypic switching [43]. VSMCs undergo from a differentiated quiescent contractile and rarely proliferate phenotype to an adverse dedifferentiated synthetic phenotype, characterized by down-regulation of contractile markers, excessive proliferation, migration and transdifferentiation to other cell types, such as fibroblasts and macrophage-like cells, a key mechanism in abnormal arterial remodeling [44]. A transcriptomic analysis of primary rat VSMCs stimulated with ANG II (100 nM , 2 h at 37°C) revealed that ANG II/ AT_1R is capable of activating multiple and synergistic signaling pathways that may be responsible for a variety of cell responses, for instance, growth and migration [45] (Fig. 2).

Through the stimulation of AT_1R , ANG II also induces the synthesis of ALDO, the major mineralocorticoid steroid hormone, secreted by the glomerulosa cells located in the cortex of adrenal gland. ALDO binds and activates the mineralocorticoid receptor (MR) [46], expressed in several cell types, including endothelial cells and VSMCs, highlighting its direct role in blood pressure control and contributing to cardiovascular physiology and pathophysiology [47,48].

The classic view of action of ALDO is to modulate renal excretory function to maintain volume homeostasis. However, several studies have shown its role in the development of inflammation, vascular

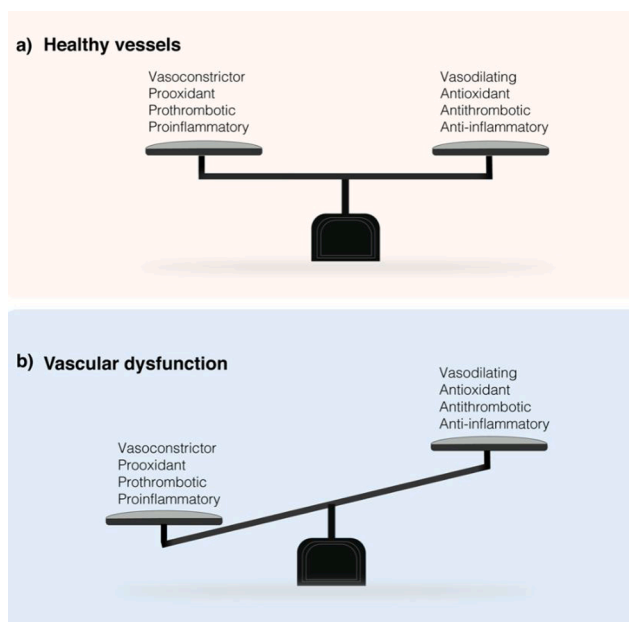


Fig. 1. Paracrine and endocrine substances affect vascular function in hypertension. (A) The balance between the production of vasoconstrictor/vasodilating, pro-/antioxidant, pro-/antithrombotic and pro-/anti-inflammatory maintains the vascular tone and the vessel's healthy (B) The imbalance between these substances leads to endothelial and vascular dysfunctions.

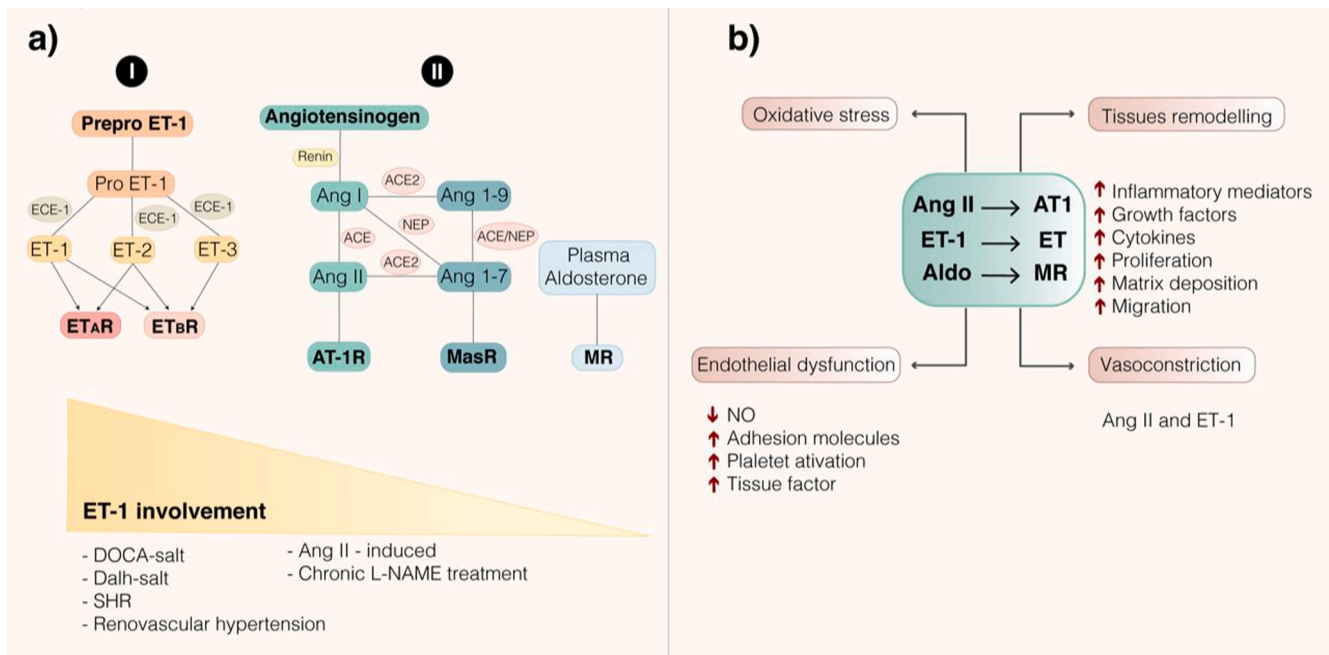


Fig. 2. a) I) Pro endothelin-1 (Pro ET-1) is converted to endothelin (ETs) under the action of endothelin converting enzymes (ECE-1). ET-1 and ET-2 activate the receptors ET_AR and ET_BR while ET-3 only the latter subtype. In several animals models such as deoxycorticosterone acetate (DOCA)- and Dalh-salt, spontaneously hypertensive rats (SHR), renovascular hypertension, angiotensin II (ANG II) infusion and in chronic L-NAME treatment the endothelin pathway is activated and involved in the deleterious effects. II) The ANG II and ANG1-7 formation encompasses three main enzymes known as angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE2), and neprilysin (NEP). ANGII and ANG1-7 activate, respectively, AT-1 and Mas receptors (R). Aldosterone (ALDO) activates the mineralocorticoid receptor (MR). b) ANG II, ET-1 and aldosterone (ALDO) when activate their receptors AT1, ET and MR, respectively, can lead to vascular dysfunction by inducing, for example, oxidative stress, inflammation, tissue remodeling, endothelial dysfunction and vasoconstriction.

remodeling and alterations in the cardio-renal-axis. Mice with deletion of MR in ~20% of renal tubule cells exhibit life-threatening weight loss, hyperkalemia and hyponatremia [49]. On the other hand, overexpression of MR in mice heart and kidneys resulted in dilated cardiomyopathy, besides no changes in blood pressure [50]. Selective deletion of MR in endothelial cells (EC-MR-KO mice) attenuates endothelial dysfunction induced by Western diet consumption (16 weeks) in adult female mice [51], whereas specific overexpression of human MR in transgenic mice endothelial cells induced higher contractile responses of mesenteric arteries to the α -adrenoceptor agonist phenylephrine, thromboxane A2 analog, ANG II and endothelin-1, without changing the vessel architecture and with a slight increase blood pressure at 4-months-old [52]. Another study observed that specific deletion of MR from the endothelium did not contribute to basal, diurnal, salt-sensitive, ALDO/salt-enhanced or ANG II-enhanced blood pressure [53]. Similarly, MR deletion in sodium-restricted female mice (0.05% NaCl, 28 days) prevented the impaired acetylcholine-induced aortic relaxation [54]. In MR-intact mice, the absence of MR did not affect the relaxation induced by acetylcholine, however, ANG II-infusion (800 ng/Kg⁻¹/min⁻¹, 2 weeks) impaired mesenteric artery endothelial-dependent relaxation, which was preserved in EC-MR-KO mice, but lost after indomethacin + L-NAME treatment, supporting the role of MR in regulating endothelial function in hypertension. Regarding contractile responses, no differences were found for mesenteric arteries from EC-MR-KO or MR-intact mice, but, interestingly, coronary vessels from EC-MR-KO mice showed reduced contractile response to endothelin-1 (ET-1) and thromboxane A2 analog, even after chronic ANG II exposure [53]. Markers of cardiac remodeling and inflammation (CCR5, iNOS, macrophages, PAI-1) are also reduced in EC-MR-KO mice treated with DOCA-salt, (8 weeks) although the increase in blood pressure was not abrogated by loss of MR in the endothelium [55] (Fig. 2b).

In mice with VSMC-selective deletion of MR have decreased blood pressure as they age (≥ 7 -month-old), in addition to reduced vascular myogenic tone and expression/activity of L-type calcium channels,

without changes in vascular structure, even though the animals were maintained with normal (0.3% NaCl), low (0.02% NaCl) or higher (6% NaCl) sodium diets [56]. Furthermore, in SMC-MR-KO infused with ANG II (800 ng/Kg⁻¹/min⁻¹, 2 weeks), the systolic blood pressure was reduced by ~31% in both aged (≥ 9 -month-old) and young (3–4-month-old) mice, accompanied by a reduction in ROS levels and ANG II-induced contraction of isolated mesenteric arteries [56]. Together, these studies showed that the activation of MR in other tissues such as in renal epithelium [49] and vascular smooth muscle [56] makes a more substantial contribution in the regulation of blood pressure, although the deletion of MR in the endothelium [53] reduced the vascular remodeling and improved the relaxation of endogenous mediators, independent of blood pressure. Vascular dysfunction resulting from chronic elevated levels of ANG II or ALDO in experimental animals is preceded by increases in markers of oxidative stress, produced in detrimental to nitric oxide (NO) bioavailability and inflammation [57,58] (Fig. 2b).

2.2. Role of endothelin-1

Endothelin (ET) is a potent vasoconstrictor, mainly produced from vascular endothelial cells, with additional mitogenic, pro-inflammatory and pro-atherosclerotic effects. Derived from its inactive precursor, preproendothelin, the formation of mature biologically active endothelin requires cleavage by the action of endothelin-converting enzymes (ECE) [59] (Fig. 2a). There are three isoforms of endothelin peptide described as ET-1, ET-2 and ET-3, encoded by three different genes, and two endothelin receptor subtypes namely ET_AR, with stronger affinity for ET-1 and ET-2 than for ET-3, and ET_BR, with equally affinity for all endothelin isoforms [60]. While both receptors are widely distributed in all tissues and organs, in humans, vascular endothelium, brain, kidneys and lungs contain more ET_BR than ET_AR, whereas ET_AR predominate in VSMCs of all blood vessels [61]. (Fig. 2a).

In VSMCs, the activation of ET_AR by ET-1 promotes contraction

[62,63], while endothelial ET_BR induces vasodilatation via the release of NO and PGI₂ [64]. Indeed, ET_BR^{+/−} knockout mice are hypertensive, possibly due to a favorable activation of the ET_AR [65] and exhibit greater vascular stenosis following injury (common carotid ligation, 14 days) than wild type mice [66].

Upregulation of the ET system is observed in several experimental models and in humans. Infusion of ET-1 (3.3 µg/Kg/h) in normotensive rats slightly increased the mean arterial blood pressure, renal vascular resistance, and sodium and water excretion, while a significant decrease in renal blood flow and medullary tissue NO was observed [67]. In healthy humans, ET-1 (2.5 ng/Kg^{−1}/min^{−1}, 2 h) also promoted a small increase in mean arterial pressure and peripheral resistance but had no significant effect on systolic blood pressure and pulse pressure [68] (Fig. 2).

Overexpression of ET-1 in the mice endothelium increases blood pressure significantly [69], while specific knockout of ET-1 in the collecting duct induces hypertension and sodium retention [70]. The endothelin system also plays a role in mediating hypertension of SHR, renovascular hypertensive rats, ANG II infused rats, DOCA-salt and Dahl-salt treated rats (for review: [28]) (Fig. 2a). In humans, circulation levels of ET-1 are usually normal in hypertensive patients, but increased in severe hypertension, heart failure, coronary artery disease, atherosclerosis, pulmonary hypertension and chronic renal disease [71]. ET-1 is also linked to the pathogenesis of hypertension by means of oxidative stress in the vascular wall [72–74] and inflammation [75,76], which are the main drivers of ED (Fig. 2b).

2.3. Role of nitric oxide/soluble guanylate cyclase pathway

Nitric oxide is a gaseous mediator with a variety of autocrine, paracrine, and endocrine effects. Nitric oxide is biosynthesized by the action of nitric oxide synthases (NOS) [77]. Once formed, it diffuses into the cell to activate its intracellular receptor – soluble guanylate cyclase (sGC), which is a heterodimer protein consisting of two subunits, alpha (α) and beta (β). The most commonly studied isoform is the α₁β₁ protein, although α₂β₁ subunits have also been identified [78]. The second

messenger cyclic 3′-5′guanosine monophosphate (cGMP) is one of the key mediators of cell signaling [79] and serves as an internal messenger to regulate a variety of physiological processes, including vascular and non-vascular smooth muscle relaxation [80–82], natriuresis [83], platelet function [84], neutrophil adhesion [85], sperm motility [86], fluid and ion secretion [87,88] and cancer cell proliferation [89] (Fig. 3).

The intracellular levels of cGMP are controlled by its formation mainly due to the activation of soluble guanylate cyclase (sGC) and particulate guanylate cyclase [90–93] and by its degradation by cyclic nucleotide phosphodiesterases (PDEs) activities [94] or its efflux to the extracellular [95,96]. The isoforms PDE5, PDE6 and PDE9 are selective for cGMP [97]. Direct sGC activation can be reached by two mechanisms: the sGC stimulators (BAY 41-2272, riociguat, vericiguat, praliciguat, BAY 41-8543, among others) are dependent on the presence of the reduced (ferrous-Fe²⁺) prosthetic iron on sGC heme and works synergistically with NO, whereas sGC activators (BAY 58-2667, BAY 60-2770 and HMR 1766) preferentially and effectively activate sGC when it is oxidized (ferric-Fe³⁺) or in a heme free state. Activating sGC has been approved for pulmonary hypertension (riociguat) [98] or acute heart failure (vericiguat) [99].

Several factors may affect the production and bioavailability of NO. Oxidative stress can cause eNOS uncoupling, a dysfunctional state of the enzyme, due to deficiency of co-factor (tetrahydrobiopterin, BH₄) or substrate (L-arginine), with the consequent production of superoxide anion (O₂^{•−}) instead of NO and then the highly reactive product peroxynitrite (ONOO[−]) [100]. Additionally, asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthesis implicated in a variety of cardiovascular diseases [101]. Higher levels of ADMA are able to induce NOS uncoupling [102,103] (Fig. 3).

2.4. Role of oxidative stress

The physiological role of ROS includes the host defense and redox-based signaling, modulating diverse aspects of cell behavior from signaling to death. The major sources involved in ROS production are

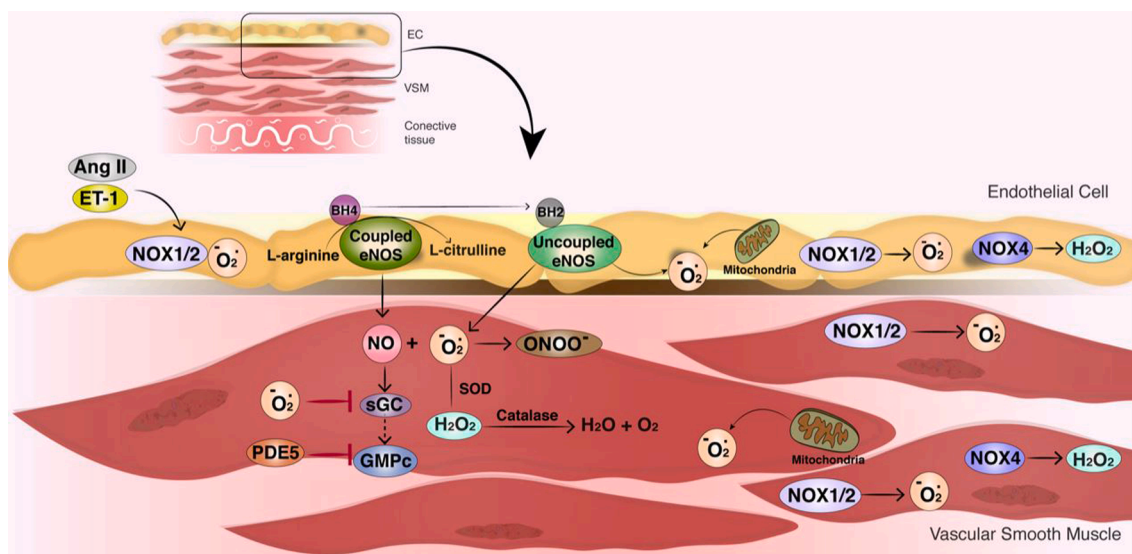


Fig. 3. The increase of intracellular calcium in response to receptor-mediated agonists or shear stress results in activation of endothelial nitric oxide synthase (eNOS), which converts L-arginine to L-citrulline and nitric oxide (NO), in a process that requires oxygen and tetrahydrobiopterin (BH₄). As a gaseous mediator, NO diffuses from the endothelium to the smooth muscle layer and activates the intracellular enzyme soluble guanylate cyclase (sGC), a heme-containing protein able to produce cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP), which in turn activates PKG, leading to a decrease of cytoplasmic calcium and relaxation of smooth muscle via several mechanisms. The effect of cGMP is mainly terminated by the action of phosphodiesterase type 5 (PDE5). The NO-cGMP pathway can be dysfunctional if the levels of NO are reduced, due to less production or peroxynitrite (ONOO[−]) formation, and/or sGC oxidized heme moiety and/or PDE5 activity is increased. Reactive oxygen species (ROS), such as superoxide anion (O₂^{•−}) and hydrogen peroxide (H₂O₂), are reduced to H₂O and oxygen through the sequential actions of superoxide dismutase (SOD) and catalase, but excessive ROS generation, often derived from mitochondria, uncoupled eNOS and NADPH oxidase isoforms (NOX), disrupts cell homeostasis and may result in vascular dysfunction. EC, endothelial cell; VSM, vascular smooth muscle.

the mitochondrial oxidases, the NADPH Oxidases (NOX) family and uncoupled eNOS [15,104–107]. Cross-talk between different sources of ROS is thought to be critical to a regenerative cycle of ROS-induced ROS release, whereby ROS formed in a subcellular compartment may trigger and enhance ROS formation in another one [108]. Deregulation of ROS production, leading to an altered oxidation-reduction (redox) state, is a common feature of several diseases, for instance, cardiovascular [109–111], renal [112], lung [113], neurodegenerative diseases [114,115] and cancer [116]. Oxidative stress has been causally linked to increased blood pressure in various experimental models of hypertension, such as SHR, DOCA-salt, Dahl salt-sensitive, 2K1C, as well as high-salt-induced hypertension (for review: [117]) (Fig. 3).

Accumulating evidence indicates that NOX is the major source of cardiovascular ROS [74,109], especially because its primary function is to produce ROS, O_2^- or hydrogen peroxide (H_2O_2) [118]. Seven members of the NOX family [NOX1, NOX2 (aka gp91^{phox}), NOX3, NOX4, NOX5, DUOX1 and DUOX2 enzymes] have been identified and their specific structure and mechanism of activation are extensively investigated (for details, see review [119]). In the vasculature, expression of NOX isoforms may vary depending on cell type, vessel size and vascular bed. Both NOX1 and NOX2 are expressed in endothelial and VSMCs, but NOX1 predominate in VSMCs. NOX3 is expressed primarily in fetal tissues. NOX4 is highly expressed in the kidney, where it was originally described [120], but is also found in humans and rodents vasculature, although is 100x more expressed in endothelial than in VSMCs [121]. NOX5 is also widely expressed in the vasculature of mammals but absent in some rodents such as mice and rats [122]. Dysregulation of NOX-dependent ROS production has been implicated in endothelial dysfunction, vessel hypercontractility *in vitro* and remodeling in various animal models of hypertension [123–125] and in atherosclerosis [110]. Furthermore, NOXs are the main sources of ROS resulting from AT₁R activation by ANG II [126] (Fig. 3).

Different NADPH oxidases may have distinct roles in regulating blood pressure and vascular cells function. Transgenic mice over-expressing NOX1 in smooth muscle cells (Tg^{SMCnoxi}, 6-month-old) exhibit a normal vascular phenotype. However, it exacerbates the hypertensive and hypertrophic responses induced by ANG II infusion (0.7 mg/Kg/day, 14 days) by reducing NO bioavailability [127], while NOX1 deletion (NOX1^{-/-}) prevented ANG II-induced alterations [128]. Aortas from NOX1^{-/-} mice exhibited reduced contractility in response to ANG II, attributed to a reduction of AT₁R activation [129]. NOX1-dependent ROS generation also regulates epithelial Na⁺ channel (ENaC) expression and activation in renal epithelial cells in response to ANG II, resulting in greater sodium and water retention, leading to increased blood pressure [130]. A twofold increase in endothelial NOX2 levels did not change basal NADPH activity, endothelial function or blood pressure. However, after both low dose (0.3 mg/Kg/day, 2 weeks) and high dose (1.1 mg/Kg/day, 2 weeks) ANG II infusion caused hypertension and greater vascular remodeling [131].

In contrast to NOX1 and NOX2, NOX4 is constitutively active, contributing to basal ROS production, and generates predominantly H_2O_2 rather than O_2^- [118]. It is known that low doses of H_2O_2 play an important role maintaining the homeostasis of vascular cells [132]. In the vascular wall, endothelial cells modulate underlying VSMC and vascular tone by releasing H_2O_2 , resulting in smooth muscle hyperpolarization and vasodilation [133]. Mice with specific endothelial NOX4 overexpression exhibit lower blood pressure and improved endothelium-dependent vasodilation [134]. At high concentrations, H_2O_2 leads to endothelial and VSMCs dysfunction and apoptosis [132]. NOX4 knockout mice have shown the reduction in oxidative stress, hypertension, cardiac hypertrophy and fibrosis in response to pressure overload or ANG II (1.1 mg/Kg/day for 14 days) infusion [135–137]. The role of NOX4 in cardiovascular dysfunction remains largely debated (for review: [138]). On one hand, a protective role against hemodynamic-overload-induced cardiac remodeling and dysfunction is well established [139], while on the other hand, contrasting results have

been reported on the role of NOX4 in ischemia/reperfusion injury, particularly in the brain [140], and neointima formation [141]. Little is known about the role of NOX4 in the pathophysiology of hypertension.

NOX5 has been largely under-researched due to its absence in experimental rodents, but transgenic mouse models express human NOX5 endogenously (hNOX5) [104]. In aged (20-month-old) hNOX5 mice, but not in young (9–15-week-old), the systolic blood pressure and mean arterial pressure were significantly increased compared to age- and sex-matched wild type mice. Both the diastolic blood pressure and the heart/body weight ratio remained unchanged indicating no cardiac hypertrophy in hNOX5 mice. Femoral arteries from aged hNOX5 showed impaired relaxation to acetylcholine in comparison to the wild-type, while preincubation with the NOS cofactor tetrahydrobiopterin (sepiapterin, 100 μ M), restored the impaired acetylcholine-induced relaxation to similar amplitude of that of the wild-type mice. Femoral arteries from aged hNOX5 also produced greater O_2^- levels and the addition of L-NAME inhibited these levels, thus suggesting that the endothelial dysfunction seen in hNOX5 mice led to eNOS uncoupling, impaired relaxation in conduit arteries and systolic hypertension [142]. Other studies corroborate the role of NOX5 in different models of hypertension [143] and stroke [144]. Furthermore, higher levels of NOX5 in endothelial microparticles from hypertensive patients have been correlated to disease severity [142]. Expression of NOX5 was also increased in cardiac tissue from patients undergoing heart transplant for cardiomyopathy and heart failure [145]. In contrast, a recent study demonstrated a potential protective role of NOX5 against atherosclerosis in rabbits [146].

DUOX enzymes are already described in the salivary and thyroid glands, lungs and intestinal tract [147], however, they have not been found or are expressed at very low levels in vascular tissue and their roles have not been established.

The NOXs emerged as potential drug-target candidates and, recently, the World Health Organization approved the stem “naxib”, recognizing NOX inhibitors as a new therapeutic class. Setanaxib (aka GKT137831) claimed as a NOX1/4 dual inhibitor and partial NOX5 inhibitor is the first naxib to reach clinical trials and is currently in phase 2 for idiopathic pulmonary fibrosis and diabetic kidney disease. However, the available compounds lack NOX isoform selectivity and most of them are pan-NOX inhibitors, exhibit ROS scavenging and/or off-targets effects and may affect physiological redox-dependent reactions [148]. Further optimization of this compounds carries substantial promises for diseases associated with NOX overactivity, e.g. hypertension, in the future.

3. Drugs that improve vascular and endothelium function in hypertension

In this section, we will prioritize results from experimental studies that explored new targets or repurposed clinically approved drugs and their effect on vascular and/or endothelial function in the context of arterial hypertension. Although the role drugs that act on the RAAS system in the context of hypertension were described previously (please read [149,150]) these classes were still explored herein because ACE inhibitors and blockers of the MR and ANG II receptors reduce the ROS or ET-1 levels and/or increase the NO levels. Besides, drugs that act on the NOX-, NO-sGC-PDE5-, ET-1- and natriuretic peptides-signaling pathways can also improve the blood pressure by reducing the deleterious effect of ANG II and/or ALDO.

Others pharmacological classes approved on the clinic settings to treat patients with hypertension such as beta-blockers, calcium channel antagonists and renin inhibitors, among others were not explored in this manuscript (please read [151–154]). When appropriate, data from clinical studies will be also explored. The RAAS inhibitors, dual angiotensin receptor-neprilysin inhibitors, ET-1 antagonists, sGC modulators, PDE5 inhibitors and sodium-glucose cotransporter 2 (SGLT2) inhibitors are explored (Table 1, Fig. 4).

Table 1

Effect of pharmacological classes in vascular function and blood pressure: preclinical and clinical findings.

Study	Phase	Drugs	Major findings
RAAS system			
Yamamoto et al. [155] Dong et al. [156]	Preclinical	Valsartan and amlodipine	Valsartan was more effective than amlodipine in i) improving ACh-induced relaxation, ii) recoupling eNOS and iii) increasing eNOS activity in Dahl SS + high-fat diet.
Chrysanthopoulou et al. [158]	Preclinical	Irbersartan	Plasma from untreated hypertensive patients increased NET release from healthy neutrophils.
			Irbersartan and DPI blocked ANG II-induced NET.
Rodrigues et al. [159]	Preclinical	Eplerenone	Hypertensive patients treated with irbersartan presented lower NET release. Mice infused with aldosterone presented higher levels of ROS and lower expression of Nrf-2. Eplerenone prevented these changes.
Sacubitril/Valsartan			
Tashiro et al. [163] Sung et al. SPS:refid::bib164[164]	Preclinical	Valsartan, sacubitril/valsartan, enalapril	Sacubitril/valsartan was more effective than valsartan or enalapril in improving the cardiac function in ANG II-induced hypertension or in female SHR rats.
Ruilope et al. [165]	Clinical	Valsartan, sacubitril/valsartan	Sacubitril/valsartan produced greater reduction than valsartan in the sitting diastolic and systolic blood pressure in hypertensive patients.
Cheung et al. [166]	Clinical	Sacubitril/valsartan, olmesartan	Sacubitril/valsartan provided greater reductions in office systolic and diastolic blood pressure and in 24-hour mean ambulatory blood pressure than the olmesartan group.
ET receptor antagonists			
Badzyska et al. [67]	Preclinical	Atrasentan, BQ788	In SHR, atrasentan reduced blood pressure, increased renal blood flow and the intrarenal NO.
Berillo et al. [170]	Preclinical	Eplerenone	Male mice overexpressing the receptor ET-1 in the endothelial layer presented higher levels of ALDO and systolic blood pressure. Esplerenone improved the relaxation induced by ACh and reduced the contraction induced by noradrenaline.
Verweij et al. [168]	Clinical	Aprocitentan	Aprocitentan was more effective than lisinopril in reducing sitting systolic and diastolic blood pressure.
NO pathway			
Stamm et al. [167]	Preclinical	Sodium nitrite	In mice infused with ANG II, sodium nitrite improved the endothelium-dependent and -independent relaxation and reduced the pro-oxidant and pro-inflammatory markers.
Yaguas et al. [169] Yugar-Toledo et al. [170]	Preclinical Clinical	Sildenafil	In SHR, sildenafil reduced systolic blood pressure and improved aorta relaxation. In hypertensive patients FMD was lower than healthy participants. Sildenafil caused a slow and progressive increase in FMD.
Stasch et al. [183]	Preclinical	BAY 58-2667	In conscious SHR, acute administration of BAY 58-2667 reduced the systolic blood pressure that lasted at least 7 h. In transgenic renin rats treated with L-NAME, the oral administration of BAY 58-2667 reduced the blood pressure.
Hanharan et al. [196]	Clinical	Pralicigat	In hypertensive and diabetic patients, pralicigat produced modest reduction in systolic, diastolic and mean arterial blood pressure accompanied by increase in the heart rate.
SGLT2i			
Hojná et al. [210] Uthman et al. [207]	Preclinical	Empagliflozin	In Ren-2 transgenic rats, empagliflozin reduced the mean arterial blood pressure. In endothelial cell, empagliflozin reduced the ROS levels.
Kravtsova et al. [211] Matthews et al. [212]	Preclinical	Dapagliflozin	In Dahl SS rats or obese mice, dapagliflozin reduced the blood pressure. In obese mice, dapagliflozin reduced the expression of tyrosine in the kidneys and the noradrenaline levels.

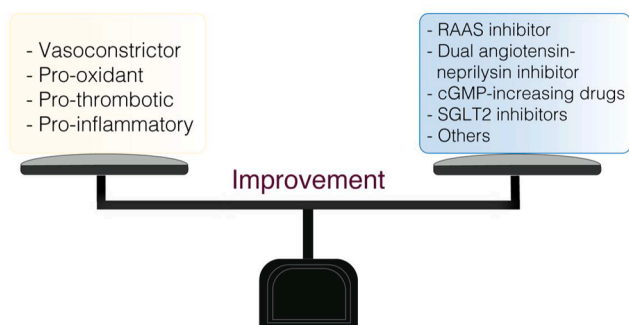


Fig. 4. Several pharmacological classes approved on the clinical settings can improve the vascular dysfunction seen in hypertension when given alone or in association by, for example, reducing the hypercontractility, proinflammatory, prothrombotic and pro-oxidant states.

3.1. Inhibitors of the RAAS system

Currently available RAAS inhibitors have proven effective in reducing blood pressure and preventing end-organ damage. The majority of studies that evaluated the role RAAS inhibitors were carried out in animal models of chronic administration of ANG II and/or ALDO with or without a loaded salt diet. However, the beneficial role of RAAS inhibition was also observed in other experimental models of hypertension such as in long-term NO inhibition and in aged SHR [28].

Concentration-response curves to endothelium-dependent substances and/or quantification of total or phospho-eNOS protein, measurement of NO or O_2^- in isolated tissues or in the endothelium and the use of markers of proliferation and apoptosis are the most common approaches used in the preclinical studies to assess the effectiveness of RAAS inhibitors. For instance, in 16-week-old male Dahl salt-sensitive (DSS) hypertensive rats, which were fed with a high-salt diet for 9 weeks old (from the 7th to 16th week), the blood pressure increased significantly at the 13th week and the treatment with the ANG II receptor antagonist valsartan (10 mg/Kg/day, 4 weeks) or the calcium channel blocker amlodipine (1 mg/Kg/day, 4 weeks) significantly reduced with similar magnitude the blood pressure in comparison with the vehicle. Despite the comparable drop on blood pressure in valsartan and amlodipine-treated groups, the former was more effective than amlodipine in preventing the impaired ACh relaxation, eNOS uncoupling and eNOS activity in DSS rats [155,156]. Another interesting finding was that in salt loaded DSS rats, the apoptosis, measured by deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), the phosphorylation of apoptosis signal-regulating kinase 1 (ASK1), a mitogen-activated protein kinase, and the ROS levels were increased in endothelial cells from salt loaded DSS rats. In valsartan-treated animals the apoptotic and oxidant processes were abrogated. In wild-type mice the infusion of ANG II increased vascular endothelial apoptosis and eNOS uncoupling, but not in ASK1^{-/-} mice, thus showing that the endothelial dysfunction caused by ANG II is, in part, mediated by ASK1, although this protein did not prevent the rise on blood pressure in mice infused by ANG II [155]. In others animal models of hypertension, the role of ASK1 protein in inducing vascular dysfunction was also explored. The increase of blood pressure induced by L-NAME (1 mg/mL, 8 weeks) was comparable in ASK1^{-/-} and wild-type mice. On the other hand, the relaxation induced by ACh in carotid artery was reduced in wild-type mice treated with L-NAME but not affected in vessels from ASK1^{-/-} mice. A decrease in eNOS uncoupling, in the apoptosis and in the ventricular weight and an increase in eNOS activity was observed in ASK1^{-/-} mice treated with L-NAME, thus showing that the deficiency of ASK1 prevents the impairment of vascular endothelial function, vascular remodeling, cardiac hypertrophy and apoptosis [157]. Similar to others studies, the deletion of ASK1 was not able to prevent the rise in blood pressure in L-NAME-treated rats. Although in the latter study the authors did not use any

inhibitor of the RAAS, the ASK1 seems to be an important pathway by which the RAAS blockers act in the cardiac and vascular dysfunction (Fig. 5a).

Innate immunity and chronic inflammation is involved in the development of atherothrombosis in patients with essential hypertension and the activation of RAAS is involved in these processes. Neutrophils isolated from healthy participants were stimulated with plasma samples from treatment-naïve hypertensive patients (48.3 ± 7.7 years old; male/female: 34/21, N = 55). A 3-fold increase in neutrophil extracellular traps (NET) was observed, thus showing that untreated hypertensive patients exhibited high plasma levels of NETosis. In isolated neutrophils from healthy participants, ANG II (0.01–100 nM) concentration-dependently increased NET release and apoptosis. The *in vitro* addition of irbersartan, the NADPH oxidase inhibitor (DPI) and wortmannin blocked ANG II-induced NETosis and autophagy. These results led the authors to conclude that ANGII enhances NET formation in a ROS/autophagy dependent manner. Interestingly, the *in vivo* findings was corroborated by *in vitro* results, as hypertensive patients under treatment with irbersartan (8 weeks after the diagnosis) presented a reduction in the NET release. These results confirm the anti-inflammatory action of inhibitors of RAAS through protection of NETosis [158].

The *in vitro* addition of ALDO (100 nM, 30 min) in thoracic aorta from male mice, impaired the relaxation induced by acetylcholine and increased the release of O_2^- and H_2O_2 . The co-treatment with eplerenone (1 μ M, 30 min), a non-steroidal MR antagonist, reversed all these changes to similar levels of those of the vehicle. Similar findings were observed in the human endothelial cells (EA.hy926), where aldosterone increased O_2^- , but not H_2O_2 , and reduced the gene expression of the antioxidant enzymes superoxide dismutase 1 (SOD1) and catalase. Eplerenone prevented ROS production in this cell line. In male mice infused with ALDO (600 μ g/Kg/min, 14 days), the isolated thoracic aorta presented higher levels of ROS and lower expression of Nrf-2 [159]. The authors did not carry out reactivity assays nor did treat the animals with eplerenone to verify whether the *in vivo* assays would reproduce the *in vitro* findings. Besides, the.

Besides, the source of ROS, whether from mitochondria and/or NOX complex, was not well explored in this study (Fig. 5a).

3.2. Dual angiotensin Receptor-Neprilysin inhibitors (ARNI)

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) possess diuretic, natriuretic and hypotensive activity. Cardiomyocytes release ANP and BNP in response to volume and pressure overload, which increase cardiac transmural distending pressure. The biologically active BNP and the non-biologically active N-terminal fragment of BNP (NT-proBNP) concentrations are valuable biomarkers in the management of heart failure since their levels raised more than ANP in left ventricular overload [160]. Both BNP and NT-proBNP are released in the plasma in equimolar quantities and the latter is an alternative choice in monitoring heart failure due to its better stability (>3 days) and longer half-life (60–120 min) when compared to BNP (24 h and 21 min, respectively) [161].

Neprilysin (NEP) is a membrane-bound zinc endopeptidase found in various organs that has many substrates, including ANP, BNP, bradykinin, calcitonin gene-related peptide, substance P, ANG II, adrenomedullin and ET-1 [162]. At first, omapatrilat, which is a combination of ACE inhibitor + NEP inhibitor, was developed, but it was failed on clinical trials due to safety concerns of angioedema. As AT₁R blockers do not interfere in the metabolism of peptides, the dual NEP- AT₁R inhibitors are available on the market to treat patients with heart failure with reduced ejection fraction. The rationale is that the downstream effects of sacubitril, a NEP inhibitor that increases natriuresis, promotes vasodilation and reduces sympathetic tone, while AT₁R blocker reduces the deleterious effects of increased ANG II.

The infusion of ANG II (3.2 mg/Kg/day, 3 weeks) in mice increased

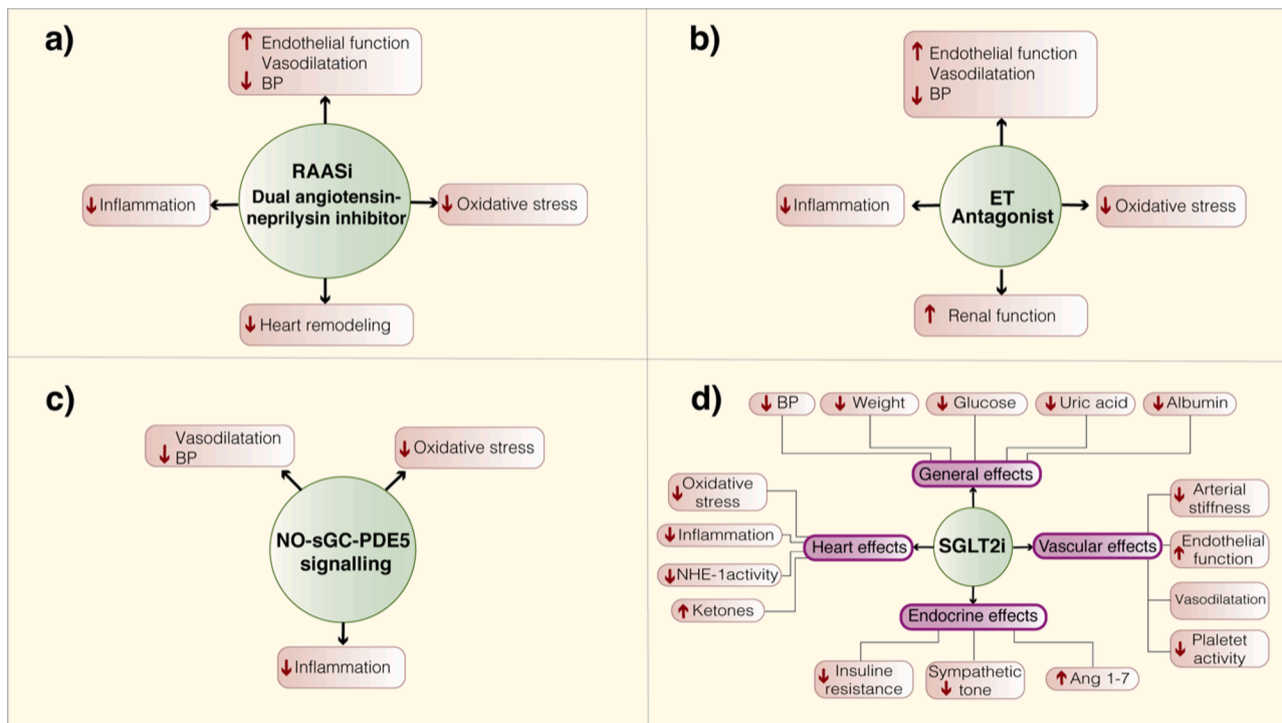


Fig. 5. Proposed pathways by which inhibitors of the renin angiotensin aldosterone system (RAASi) and dual angiotensin receptor-neprilysin inhibition (ARNI), antagonists of the endothelin-receptor, inhibitors of phosphodiesterase type 5 (PDE5), NO-donors or soluble guanylate cyclase (sGC) modulators, and sodium-glucose transporter 2 inhibitors (SGLT2i) can improve the hypertensive state beyond blood pressure (BP) reduction. NHE-1, sodium-hydrogen antiporter-1.

both systolic and diastolic blood pressure, and the treatments with sacubitril/valsartan (60 mg/Kg/day, gavage) or valsartan (30 mg/Kg/day, gavage) or enalapril (12 mg/Kg/day, gavage) for 2 weeks produced a modest decrease in the blood pressure, measured by tail cuff. Sacubitril/valsartan significantly suppressed ANG II-induced increase in interventricular septum thickness diameter, left ventricular posterior wall thickness diameter and left ventricular wall thickness. The increase in cardiomyocyte size induced by ANG II were reduced by all the three treatments, although, the inhibitory effect of sacubitril/valsartan was greater than valsartan- and enalapril-treated mice. The authors did not discuss whether the dose chosen was similar to the matching doses approved on the clinic [163]. In female SHR (18-week-old), valsartan (160 mg/Kg) or sacubitril/valsartan (300 mg/Kg) significantly reduced systolic, diastolic and mean arterial blood pressure, although, the magnitude of inhibition was significantly greater in sacubitril/valsartan group. In anaesthetized animals, sacubitril/valsartan was more effective than valsartan in reducing the relative wall thickness and the iso-volumetric relaxation time. The ventricular arrhythmia and heart rate were moderate in SHR treated with sacubitril/valsartan than valsartan. These effects were accompanied with lower fibrosis area and lower plasma levels of NT-proBNP, thus showing that in hypertensive rats the treatment with sacubitril/valsartan were more effective in reduce cardiac hypertrophy and ventricular arrhythmias [164].

Patients with mild hypertension (18–75 years) were randomly assigned to receive for 8 weeks sacubitril/valsartan, valsartan or placebo. The magnitude of reduction in the sitting diastolic and systolic blood pressure was greater in those participants who received sacubitril/valsartan (200 and 400 mg) than in the valsartan group (160 and 320 mg). The doses of the valsartan group was chosen based on a previous pharmacokinetics studies where they produced similar systemic exposure of that of sacubitril/valsartan [165]. In phase III trial, multicenter, randomized, double-blind, parallel-group and active-controlled trial involving patients with essential hypertension uncontrolled by olmesartan (20 mg/day), sacubitril/valsartan provided greater reductions in office systolic and diastolic blood pressure and in 24-hour

mean ambulatory blood pressure (baseline to week 8) than the valsartan group. At the screening period, patients who were receiving olmesartan entered in a washout period of 1 to 2 weeks [166].

3.3. Endothelin-1 receptor antagonists

Although endothelins are involved in several conditions including respiratory distress syndrome and cardiovascular diseases, to date the antagonists named bosentan, ambrisentan and macitentan are only approved to treat patients with idiopathic pulmonary arterial hypertension [71]. Preclinical trials showed the beneficial effects of endothelin antagonists in reducing the blood pressure and target-organ damage, but clinical trials continue to explore new applications of these antagonists in patients with hypertension and chronic kidney disease [167,168].

The ET_AR antagonist, atrasentan (0.6 mg/Kg/h, iv), but not the ET_BR antagonist BQ788, improved renal hemodynamics of male SHR (12-weeks-old) by decreasing the blood pressure, increasing renal blood flow, urine flow and a tendency of decrease of sodium excretion, in addition to increasing intrarenal NO bioavailability, thus suggesting that in SHR the endothelin-system is overactivated [67]. The acute pressure-natriuresis induced by ET-1 and its blockade by the ET_AR antagonist was also confirmed previously in normotensive rats [169]. An elegant study has observed the possible crosstalk between ALDO and ET-1. In male mice (3-months) overexpressing human ET-1 restrictly in the endothelium layer (eiET-1), higher levels of ALDO and lower sodium excretion were presented. Furthermore, eiET-1 mice exhibit a rise on blood pressure in a mineralocorticoid-dependent manner, as elevated systolic blood pressure, measured by telemetry, was significantly reduced by the oral treatment with eplerenone (100 mg/Kg, 14 days) during the daytime, but not during the nighttime. In eplerenone-treated mice, the hypercontractility induced by noradrenaline and the lower relaxation induced by acetylcholine were significantly improved [170]. The dose of eplerenone was chosen based on its effectiveness in blocking the rise of blood pressure induced by aldosterone and it is not discussed whether it

is in the range of a therapeutic dose. Previous studies had already shown that atrasentan also improved the sustained blood pressure elevation, greater vascular reactivity to catecholamines and vascular remodeling in e1ET-1 mice [171,172] (Fig. 5b).

Overactivation of endothelin system has emerged as a potential contributor to hypertension and glomerular kidney damage induced by vascular endothelial growth factor (VEGF) inhibition [173,174]. Cola-fella and colleagues [174] showed that sunitinib-induced hypertension in rats, tyrosine kinase receptor inhibitor, was mediated by the activation of ET_AR, accompanied by greater ROS and cyclooxygenase (COX)-2-dependent byproducts production. These effects were completely prevented by the administration of the ET_AR antagonist, sitaxentan (100 mg/Kg/day for 8 days) or the dual ET_{A/B}R antagonist, macitentan (30 mg/Kg/day for 8 days).

The antihypertensive effect of the dual endothelin receptor antagonist, apocritentan was observed in hypertensive patients. In a randomized, double-blind, multicenter, placebo and active comparator-controlled trial, apocritentan (10, 25 and 50 mg) significantly reduced the sitting systolic and diastolic unattended automated office blood pressure from baseline to week 8 in comparison the lisinopril group (20 mg). Similar findings were observed in mean 24-hour systolic/diastolic blood pressure [168].

3.4. Soluble guanylate cyclase modulators and phosphodiesterase type 5 inhibitors

The improvement in the NO levels can be achieved, for example, by inhibiting NOX and/or recoupling the eNOS. In the aorta from aged SHR (12–14-month-old) a greater level of O₂ and higher NOX activity were observed. The *in vitro* incubation with antioxidants, Tyron and apocynin, and the pan-NOX inhibitors, diphenylene iodonium (DPI) and VAS2870, but not L-NAME or oxypurinol, blocked the increased signal in aorta. The protein levels of NOX1 (media and intima) and NOX2 (adventitia and intima), but not NOX4, was increased in the aorta from aged SHR. Both VASP2870 and apocynin improved ACh-induced relaxation in aorta from SHR [175]. In this study, the authors did not treat the animals to verify whether VASP2870 or apocynin per se could improve blood pressure levels nor did carry out functional assays in resistant arteries. As described above, both ANG II and ALDO increase ROS levels in the heart, kidney, vessels and their blockade by ACE inhibitors, AT₁R and MR blockers improve vascular smooth muscle and the endothelial function. However, to date, there are no therapies approved on the clinic that directly target NOX or eNOS recoupling for the treatment of vascular-related diseases.

Organic nitrates can be used for acute therapy of hypertensive crisis or in patients with acute or chronic heart failure. Nevertheless, they are not effective for long-term use in hypertension due to tolerance and side effects [176,177]. The inorganic nitrite (NO₂) and nitrate (NO₃) obtained from vegetables leads to an increase in NO levels to produce antihypertensive effects. The first human evidence of nitrate-leading to lower blood pressure was observed in 2006 [178].

In mice infused with ANG II (1 mg/Kg/day, 7 days) and treated with sodium nitrite (7.5 mg/Kg/day) or sodium nitrate (150 mg/Kg/day), only sodium nitrite improved the systolic and diastolic blood pressure, the endothelial-dependent and -independent relaxation, the oxidative stress measured by anion superoxide in the aorta and heart and the inflammatory markers. Although there are evidences about the beneficial effect of nitrate in ameliorating blood pressure, it was only observed in high doses and in long-term treatment (>2 weeks) [179]. The study in rodents on nitrate and nitrite has limitations to translate into clinical settings because the entero-salivary bioactivation pathways may differ between rodents and humans. In hypertensive.

patients dietary nitrate (250 mL of beetroot juice, 4 weeks) reduce the systolic and diastolic blood pressure measured in the clinic and on 24-hour ambulatory in comparison with the placebo [180] (Fig. 5c).

PDE5 inhibitors have been shown to exert mild transient peripheral

vasodilatory and hypotensive effects. In young male SHR rats (13 weeks) treated by 6 months with sildenafil (2.5 mg/Kg, oral gavage), systolic blood pressure was reduced by approximately 25 mmHg, while no alterations were observed in the age-matched control group. In aorta from sildenafil-treated rats, the hypercontractility induced by phenylephrine and the impaired relaxation induced by acetylcholine were reversed to similar levels of the normotensive rats. The finding that sildenafil improved the relaxation induced by acetylcholine does not mean that the endothelial function was improved, as stated by the authors. No assay was carried out to using markers of improvement in the endothelial function. As sildenafil inhibits PDE5, the greater relaxation induced by acetylcholine was due to the amplification in the NO-cGMP signaling pathway. Additionally, it was not clear how the dose of 2.5 mg/Kg was converted into a dose used on the clinic [181]. The vasodilatory effect of sildenafil was assessed in healthy and in refractory hypertensive patients through FMD technique. FMD was lower in hypertensive than in healthy volunteers in the absence and after the NO-donor administration, thus suggesting an impairment in the NO-signaling pathway. Sildenafil (50 mg) caused a slow and progressive increase in FMD in both the healthy and hypertensive patients. In this study the authors did not assess the blood pressure [182] (Fig. 5c).

One of the first evidence that sGC activator interfere in the blood pressure was done by the researchers Peter Stasch and Harald Schmidt. In anesthetized rats, BAY 58–2667 (10 µg/Kg, iv) produced a long-lasting reduction in the blood pressure (maximal effect 29 mmHg) and this response was even greater (71 mmHg) in animals pre-treated with the sGC oxidant, ODQ (2 mg/Kg, iv, 10 min before BAY 58–2667). In conscious male SHR (age not specified), the acute administration of BAY 58–2667 (3 mg/Kg, oral) reduced the blood pressure that lasted around 7 h. In a 11-week old transgenic renin rats (TG(mRen2)27 treated with L-NAME, the systolic blood pressure, measured by tail cuff, increased significantly systolic blood pressure (159 to 176 mmHg) and the treatment with BAY 58-2667 (10 mg/Kg, oral gavage, bid, 5 weeks) reduced by 150 mmHg. Plasma levels of BNP, renin, creatine and urea was lower in BAY 58-2667-treated rats [183]. Other studies have corroborated this previous finding showing the vasodilating effect of sGC modulators (stimulator and activator) *in vitro* and *in vivo* in both acute and chronic treatment in experimental models of hypertension [184–192]. The studies that used mice that lack sGC subunits or its downstream protein kinase G (PKG) also showed impairment in the vascular function thus leading to a hypertensive state. The redox state of the heme portion of sGC can be modulated by cytochrome b5 reductase 3 (CYB5R3), which maintains the iron in its reduced state (Fe²⁺). In mice that did not express CYB5R3 on the smooth muscle layer (SMC CYB5R3-KO), the mean arterial blood pressure, measured by 24-hour telemetry, was increased significantly by 5.8 mmHg in comparison to the age- and sex- match control without affecting the heart rate. As ANG II can oxidize sGC subunits through ROS increase, in SMC CYB5R3-KO mice infused with ANG II (750 ng/Kg/min, osmotic pump, 14 days) a significant rise on the mean arterial blood pressure (14 mmHg) and heart rate were observed, which was prevented by the infusion of BAY 58–2667 (0.04 mg/Kg). Taken together, this study proposed that impairment in the sGC subunits produced a more aggressive hypertension in animals that received ANG II [193]. Other study have shown that the lack of sGC subunits in mice led to higher blood pressure [194].

In humans, genome-wide association study identified a genetic variant in the *GUCY1A3-GUCY1B3* locus (encoding sGCα₁ and sGCβ₁, respectively), which was associated with hypertension, left ventricular wall thickness, stroke, and coronary artery disease [195]. In diabetic and hypertensive patients under stable glucose and hypertension therapy, pralicigat (40 mg/day, 14 days) produced greater systolic, diastolic and mean arterial blood pressure reduction, although modest, accompanied by an increase in the heart rate [196]. In a multicenter, randomized, double-blind, parallel group, placebo-controlled, phase 2 trial, the sGC stimulator pralicigat (20 and 40 mg, 12 weeks) did not improve significantly the renal function. A significant decrease, although modest

(4 mmHg), was observed in the mean 24-hour systolic blood pressure in diabetic patients with kidney disease in comparison with the placebo arm [196]. In both studies, pralicigat improved the metabolic parameters.

Oxidation of the sGC subunits or higher expression of PDE5 can also be altered thus contributing to greater peripheral resistance and hypertension. Although this pathway is one of the most studied in the vascular-related diseases, the chronic use of organic nitrates or sGC stimulator or PDE5 inhibitors alone or in combination are not approved as first line monotherapy or combined therapy in patients with hypertension. The effectiveness of sGC modulators and PDE5 inhibitors are strongly reduced in situations where NO are reduced *in vitro* [197] or in chronic inhibition of NO [198] and it can be one plausible explanation by which these classes are not effective as monotherapy as, for example, diuretics, RAAS blockers and calcium channel blockers. Besides, as symptomatic hypotension is an adverse effect commonly seen in the clinical trials with the sGC stimulators used on the clinic (riociguat or vericiguat), more clinical trials are needed with patients with essential or resistant hypertension in order to assess the efficacy and therapeutic dose regimen on drugs that act on NO-sGC-PDE5 signaling pathway.

3.5. Sodium-glucose cotransporter-2 inhibitors

Sodium-glucose cotransporter-2 (SGLT-2) inhibitors, also known as gliflozins (empagliflozin, dapagliflozin, canagliflozin, ipragliflozin, luseogliflozin, topogliflozin), were initially approved on the clinical to treating patients with type 2 diabetes, as they decrease renal glucose reabsorption in the proximal tubule by up to 50% with lower risk of inducing hypoglycemia [199]. Beyond their glucose-lowering effects, all gliflozins also exhibits multiple pleiotropic effects, particularly related to the improvement in the cardiovascular system (for review see [200]), thus showing that this is a class effect. In three randomized controlled trials known as CANVAS [201], DECLARE-TIMI [202] and EMPA-REG [203] trials, type 2 diabetes patients who received gliflozins presented lower rate of cardiovascular death or hospitalization for heart failure and prevented the progression of renal dysfunction who were already receiving guideline-directed medical therapy. Due to these beneficial cardiovascular outcomes, gliflozins were repurposed to treat patients with heart failure and/or chronic kidney disease in patients with or without [45]. The reduction in blood pressure induced by gliflozins was observed mostly on sub-analysis of the clinical trials and in real-world trials (a bedside-to-bench evidence) in patients already taken anti-hypertensives. Weight loss [204,205], increase natriuresis and diuresis [206], inhibition of the antiporter Na^+/H^+ in myocardial cells [207] and improvement in the metabolic variables [208] are some of the proposed mechanisms by which this class of drugs could reduce the blood pressure [209] (Fig. 5d).

In a 6-months old, non-diabetic hypertensive model (Ren-2 transgenic rats [TGR]), a significant increase in the systolic and diastolic blood pressure were observed along with signs of metabolic syndrome, as characterized by increase body weight and perirenal adipose tissue weight and insulin resistance, although the non-fasting glucose was not changed. The treatment with empagliflozin (10 mg/Kg/day) for 6 weeks significantly reduced the mean arterial blood pressure in awake hypertensive rat from the fourth week reaching a maximum drop of 20 mmHg (day and night) at the end of the sixth week. As expected, both the sodium and glucose excretion was increased in empagliflozin-treated rats, reaching its peak after 4 weeks of treatment. The loss of energy in the form of glucose, the natriuresis and the direct vasodilation can be possible explanations by which empagliflozin reduced the blood pressure in these animals. Conversely to others studies, empagliflozin did not improve the heart and kidney function, however, one possible explanation was that this study was carried out in middle-aged rats (6-months old) and/or the duration of treatment, which did not present yet a strong target organ lesion. A more pronounce effect in the cardio-renal axis would be seen in aged rats (>2 years old) [210]. Chronic inhibition of

SGLT2 with dapagliflozin (2 mg/Kg/day, 3 weeks in drinking water) effectively attenuates the development of hypertension in DSS rats, through glucosuria and natriuresis without changes in the RAAS system or activity of the main sodium channels and transporters [211].

In obese mice treated with dapagliflozin (40 mg/Kg/day, 10 days) the mean arterial blood pressure and fasting glucose were reduced. The protein expression of SGLT2 was increased in the kidney from dapagliflozin-treated obese mice. On the other hand, the expression of tyrosine hydroxylase and the levels of noradrenaline were increased in the kidney and heart from obese mice, whereas the treatment with dapagliflozin reduced these levels, thus suggesting that the cardio-renal protection induced by gliflozins may be in part due to a reduction in the sympathetic activity [212]. Canagliflozin concentration-dependent relaxed isolated arteries from visceral adipose tissue of either non-obese or obese humans, which was unaffected by the endothelial removal, NOS or COX inhibition, thus ruling out the involvement of NO or prostanoids in canagliflozin-induced relaxation [213]. Other study corroborates the previous findings showing that the relaxation induced by dapagliflozin in rabbit aorta involves the direct activation of protein kinase G but was not affected by the endothelial removal [214]. Although gliflozins seem not to affect vascular reactivity in an endothelium-dependent manner, others studies observed an anti-inflammatory and antioxidant role of gliflozins in the endothelial cells. In healthy endothelial cells from human umbilical vein or coronary arteries, TNF-alpha increased the activity of sodium-hydrogen antiporter 1 (NHE-1), ROS generation. Previous incubation with empagliflozin (1 μM , 30 min) reduced ROS levels through NHE-1 inhibition and lower intracellular levels of sodium [207]. The anti-inflammatory role of gliflozins was conducted in healthy immortalized endothelial cells under static conditions, which might not reflect the ED presented in patients with hypertension with or without comorbidities. Additionally, platelets reactivity obtained from healthy volunteers were significantly reduced when canagliflozin or empagliflozin or dapagliflozin we co-incubated *in vitro* with the endothelial mediators, NO or prostacyclin, thus suggesting that it could be another mechanism of action by which gliflozins improve the cardiovascular axis [215] (Fig. 5d).

Although the preclinical data showed a reduction in the blood pressure and a prevention in target organ damage in animals that received gliflozins as monotherapy, care should be taken to translate these data into clinical settings, as in the majority of clinical studies patients were already taken other antihypertensive drugs that are known to improve the cardiovascular outcomes. Therefore, it is not yet clear whether gliflozins can be repurposed as monotherapy in hypertensive patients without chronic kidney disease, heart failure or diabetes or should be used in combination with others classes in order to enhance the blood-pressure lowering effects and the cardio-renal protection.

4. Final remarks

Vasoactive stimuli promoted by catecholamines, ANG II, ALDO and ET-1 and among others as well as mechanical forces and physical factors are often the pivotal players, as they stimulate numerous downstream signaling pathways, thus being considered as useful targets for intervention in hypertensive patients. The NO pathway is a key determinant of endothelial function and vascular health, underlying some of the blood pressure-lowering effects of currently used cardiovascular drugs. Currently, attention is also focused on the activity of NADPH oxidases as a critical determinant of the redox state of blood vessels.

However, considering the expression “from bench to bedside” we conclude that therapeutic alternatives to treat patients with arterial hypertension did not immediately follow biochemical and pathophysiological revolution. The last approved pharmacological classes to treat patients with hypertension are the ANG II receptor in the 1990's and renin inhibitors in the 2000's, although the latter class is not even considered first therapeutic option. There are several plausible drawbacks to translate the preclinical data into the clinic such as: isogenic or

genetically modified animals housed in a clean and in a silent environment, age of the animals, animal species, type of hypertension model, the duration of the hypertension, presence or not of target-organ lesions, the antihypertensive dose, the techniques to assess target organ damage, blood pressure measurement techniques (anaesthetized, tail-cuff, telemetry, acute or 24-hour) and the lack of understanding about the molecular mechanisms underlying the dysfunctions seen in hypertension. Drug repurposing is an attractive area. However, further preclinical and translational studies are needed to assess the mechanistic pathways involved in essential hypertension and the identification of the right drug.

CRedit authorship contribution statement

Mariana Gonçalves de Oliveira: Writing – review & editing. **Wilson Nadruz:** Writing – original draft, Writing – review & editing. **Fabiola Zakia Monica:** Conceptualization, Writing – original draft, Writing – review & editing, Resources, Supervision, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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