Endothelial factors in the pathogenesis and treatment of chronic kidney disease Part I: General mechanisms: a joint consensus statement from the European Society of Hypertension Working Group on Endothelin and Endothelial Factors and The Japanese Society of Hypertension

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Kidney damage is a common consequence of arterial hypertension, but is also a cause of atherogenesis. Dysfunction and/or harm of the endothelium in glomeruli and tubular interstitium damage the function of these structures and translates into dynamic changes of filtration fraction, with progressive reduction in glomerular filtration rate, expansion of extracellular fluid volume, abnormal ion balance, and hypoxia, ultimately leading to chronic kidney disease. Considering the key role played by endothelial dysfunction in chronic kidney disease, the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension and the Japanese Society of Hypertension have critically reviewed available knowledge on the mechanisms underlying endothelial cell injury. This resulted into two articles: in the first, we herein examine the mechanisms by which endothelial factors induce vascular remodeling and the role of different players, including endothelin-1, the renin–angiotensin–aldosterone system and their interactions, and of oxidative stress; in the second, we discuss the role of endothelial dysfunction in the major disease conditions that affect the kidney.

Keywords: artery, atherosclerosis, blood pressure, diabetes mellitus, endothelin, endothelium, hypertension, kidney, nitric oxide, renal failure

Abbreviations: ACE, angiotensin I-converting enzyme; ADMA, asymmetric dimethylarginine; Ang II, angiotensin II; ARB, angiotensin AT_1 receptor blocker; BP, blood pressure; CKD, chronic kidney disease; ECE, endothelin converting enzyme; EDH, endothelium-derived hyperpolarization; eGFR, estimated glomerular filtration rate; EMT, epithelial to mesenchymal transition; ENaC, epithelial or endothelial Na^+ channel; eNOS, endothelial nitric oxide synthase; ERA, endothelin receptor antagonist; ESRD, end-stage renal disease; ET-1, endothelin-1; GFR, glomerular filtration rate; GPER, G-protein-coupled estrogen receptor; HIF-1, hypoxia-inducible factor-1; iNOS, inflammatory nitric oxide synthase, NOS 2; L-NMMA, N(G) monomethyl-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; PIP_3, phosphatidyl-inositol (3,4,5) trisphosphate; RAAS, renin–angiotensin–aldosterone system; ROS, reactive oxygen species; VSMC, vascular smooth muscle cells

INTRODUCTION

Arterial hypertension is the most prevalent modifiable cardiovascular disease risk factor and contributes significantly to global disease burden [1,2]. It is often associated with chronic kidney disease (CKD), which

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is also an independent risk factor for atherogenesis [3], an important determinant of overall cardiovascular risk [4,5]. Renal vasoconstriction and/or impaired Na^+ excretion are hallmarks of the early stages of both hypertension and CKD, thus implicating a key role for the kidney in the development of hypertension. CKD patients are at increased risk of hypertension, coronary artery disease, and stroke, which are all major causes of death in these patients even before the development of end-stage renal disease (ESRD) [6]. Thus, the CKD is both a cause and consequence of hypertension [7].

For decades, the endothelium was regarded as an inactive layer lining the vessels [8]. However, after the pioneering work of many investigators, including the Nobel laureates Furchgott [9], Ignarro [10], and Murad, who discovered the endothelium-derived relaxing factor nitric oxide (NO), and Vanhoutte and de Mey [11], who described the endothelium-independent vasoconstriction, Hickey *et al.* [12] first reported an endothelium derived vasoconstrictor activity [12], which was subsequently identified as endothelin-1 (ET-1) by Yanagisawa and Masaki [13], it soon became clear that the endothelium is key for the preservation of vascular integrity. In effect, with a weight in an adult man similar to that of the liver and a surface area throughout the body comparable with that of four tennis courts, it represents the largest organ in the body [8].

Impaired endothelium-dependent vasodilation is a hallmark of many cardiovascular disease risk factors and conditions apart from hypertension, suggesting that it can be an early mechanism leading to cardiovascular damage or, alternatively, a marker of it [14]. Endothelial dysfunction, which involves the entire vascular tree and implies a shift toward a proinflammatory prothrombotic state [15], also affects the renal vasculature. Given the specific function of glomeruli and tubules (e.g. plasma ultrafiltration, ion reabsorption, and cell acidification), endothelial loss and/or dysfunction translates into dynamic changes of filtration fraction, resulting in a progressive reduction in the glomerular filtration rate (GFR), extracellular fluid volume expansion, abnormal ion balance, and renal hypoxia, all of which ultimately lead to CKD.

Recognizing the key roles played by the endothelium in cardio-renal health, as well as the lack of up to date information on its role in CKD, the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension in conjunction with the Japanese Society of Hypertension prepared this consensus document to summarize current knowledge of the mechanisms underlying endothelial cell injury and its role in renal damage in arterial hypertension, diabetes mellitus, preeclampsia, kidney transplantation, and cancer patients undergoing antiangiogenic therapy. Part I is focused on the general mechanisms underlying endothelial dysfuntion in the kidney; Part II will discuss the role of the endothelium in disease conditions affecting the kidney.

Methodology and literature search

Each section was assigned to one or two authors, who were responsible for the initial search and selection of the retrieved articles. Each author searched the PubMed and Google Scholar databases (http://libguides.mit.edu/c.php?g=175963&p=1158594) for knowledge generated within these fields over the past decade using the MeSH terms reported in Supplemental Table 1, http://links.lww.com/HJH/A865. Only articles written in English language were considered. Apart from the articles identified by such strategy, the reference lists of previous reviews published on this topic were examined. Seminal articles, even though published more than one decade ago, or review articles were quoted if considered fundamental for the present review. Criteria used for selection of the retrieved articles were appropriateness of methodologies; novelty; relevance for understanding mechanisms and/or for clinical practice; expertise of the authors in the field; source of publication. Then, to offer a coherent narrative rather than a descriptive review, the article was reviewed by each other author, who also critically evaluated the cited literature. A thematic cross-disciplinary (internal medicine, nephrology, pharmacology) approach was chosen to avoid a bias toward a specific discipline.

ENDOTHELIUM-DEPENDENT MECHANISMS AND KIDNEY INJURY

Mechanical stress, hypoxia, aging, smoking, hypercholesterolemia, diabetes mellitus, and hyperhomocysteinemia all lead to an increased formation of reactive oxygen species (ROS), which inactivate NO and increase the formation of vasoconstricting and mitogenic substances. This results in an imbalance between vasodilating and vasoconstricting endothelial factors (Fig. 1). A decreased bioavailability of NO and/or an increased release of NO inhibitors, such as asymmetric dimethylarginine, reduce NO bioactivity on the renal vascular smooth muscle cells (VSMC), blunting vasodilation (Fig. 1) [16–18]. A reduced release of factors causing endothelium-derived hyperpolarization (EDH) (like epoxyeicosatrienoic acids) reduces K^+ channels activity and promotes Ca^{2+} influx, thus activating VSMC contraction (Fig. 1). When both the release of vasodilators and endothelium-derived hyperpolarization (EDH) are blunted, vasoconstrictors as, angiotensin II (Ang II) and ET-1 act unopposed to induce renal and systemic vasoconstriction. This leads to reduced renal blood flow and hypoxia, with ensuing activation of the renin–angiotensin–aldosterone system (RAAS) [19]. The negative feedback mechanism whereby Ang II suppresses renin release might modulate this RAAS activation, but the effectiveness of this counterregulation in different clinical settings remains to be established.

Moreover, hypoxia inhibits NO synthesis and facilitates formation of hypoxia-inducible factor-1 and ROS, which, via inflammatory NO synthase (initially referred to as inducible NOS), promote generation of large amounts of NO in leukocytes, VSMCs, and epithelial tubular cells. This leads to the formation of peroxynitrite (ONOO), which aggravates microcirculatory dysfunction [20], and possibly also to destabilization of atherosclerotic plaques, as ONOO activates matrix metalloproteinases (Fig. 1) [21].

The production of the potent vasoconstrictor ET-1 is triggered by multiple stimuli, including Ang II, ROS, and

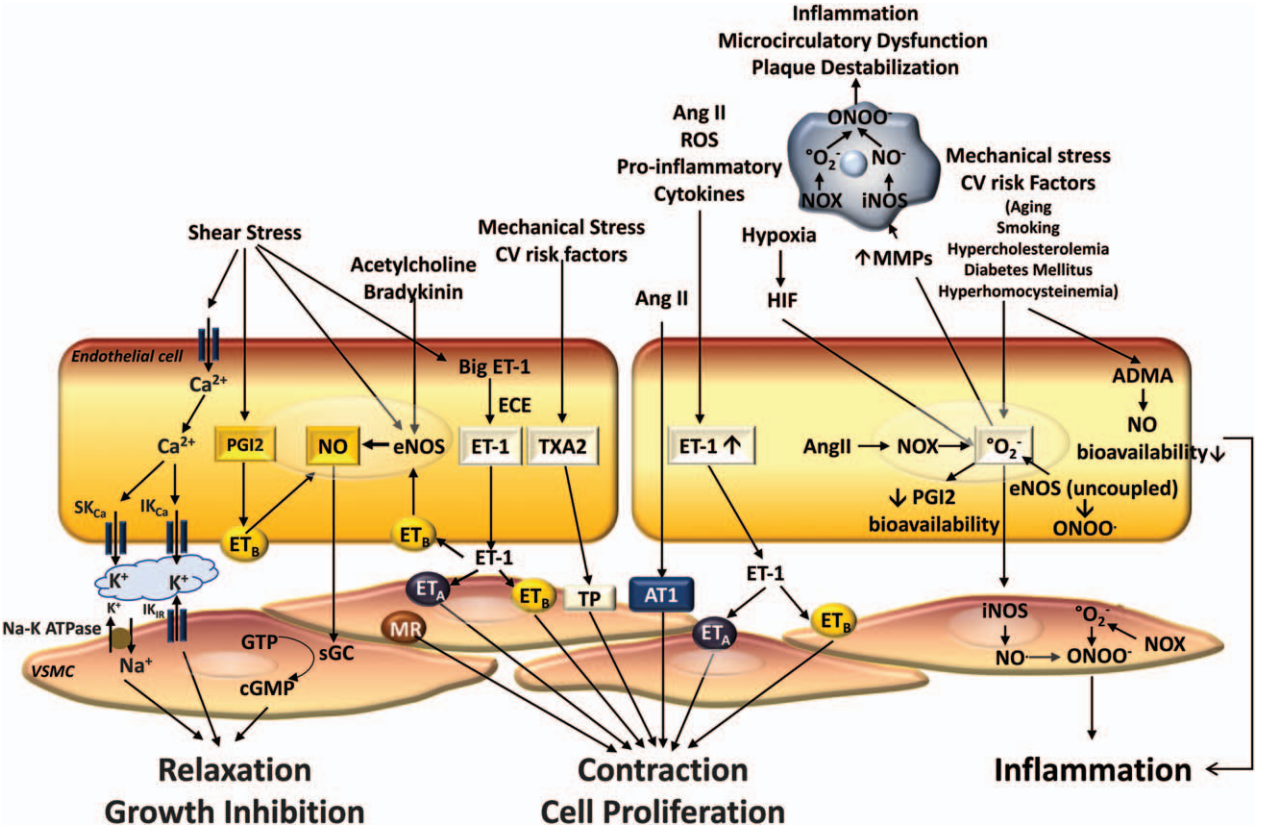

FIGURE 1 Endothelium-derived factors inducing relaxation/growth inhibition and contraction/cell proliferation of vascular smooth muscle cells. Vasodilatating factors include nitric oxide, PGI2, and endothelium-derived hyperpolarization, whereas contracting factors include endothelin-1 and endothelium-derived contracting factors, including TXA and prostaglandin H2. Shear stress, acetylcholine, and bradykinin activate endothelial nitric oxide synthase, which generates the soluble gas nitric oxide that, after diffusing through the cell membranes of endothelial and vascular smooth muscle cells, binds the soluble guanylate cyclase. This latter synthesizes cyclic guanosine monophosphate from guanosine-5′-triphosphate, thereby inducing vascular smooth muscle cell relaxation. In contrast, relaxation triggered by endothelium-derived hyperpolarization is mostly mediated by activation of K^+ channels and blunting of Ca^{2+} channels activity. The efflux of K^+ through endothelial IKCa and SKCa result in a localized 'K^+ electron cloud', which in turn stimulates hyperpolarization of the underlying vascular smooth muscle cells. Endothelin-1 is generated from big endothelin-1 via two endothelin converting enzyme isoforms, endothelin converting enzyme-1 and endothelin converting enzyme-2. In vascular smooth muscle cell, endothelin-1 exerts contracting and growth-promoting effects primarily via ET_A receptors (and in some vascular beds also via ET_B receptors), whereas when it binds ET_B receptors in endothelial cells, activates endothelial nitric oxide synthase leading to nitric oxide production, finally inducing relaxation. EDCF (predominantly the cyclooxygenase-derived EDCFs TXA_2 and PGH_2) and angiotensin II cause vascular smooth muscle cells contraction and proliferation via prostanoid (TP) and AT_1 receptors, respectively. Under physiological condition, a balance between relaxation and contraction allows maintenance of vascular tone. Excess production of angiotensin II and cytokines, or release of reactive oxygen species during inflammation and hypoxia, favors production of endothelin-1 and hypoxia-induced factor along a decrease of nitric oxide, thereby causing an unbalanced vasoconstriction. Excess mechanical stress, as well as cardiovascular risk factors, as aging, smoking, hypercholesterolemia, diabetes mellitus, and hyperhomocysteinemia, also favor production of reactive oxygen species, particularly superoxide anion ($^{\circ}O_2^-$), which by reacting with nitric oxide as the diffusion limited rate ($6.7 \times 10^9 \times s^{-1}$) forms peroxynitrite ($ONOO^-$), thereby inactivating nitric oxide. ADMA also decreases nitric oxide bioavailability and activate metalloproteases that, in turn, induce $ONOO^-$ production in mononuclear cells, finally amplifying inflammation and leading microcirculatory dysfunction and plaque destabilization. GMP, guanosine monophosphate; MAPK, mitogen-activated protein kinase; NF-kB, nuclear factor kappa beta; ONOO, peroxynitrite; PGI, prostacyclin; TP, prostanoid; TXA_2, thromboxane A_2.

proinflammatory cytokines [22]. Under pathophysiological conditions, this can take place in renal cells other than the endothelial cells, including podocytes, parietal epithelial cells, and mesangial cells [23] (Fig. 2). Endothelial cells secrete ET-1 predominantly toward the abluminal side, in which endothelin type A and B (ET_A, ET_B) receptors (under disease conditions) are located, thereby triggering vasoconstriction and cell proliferation. Thus, ET-1 induces contraction and growth by acting in a paracrine fashion on VSMCs. ET-1 also releases NO and prostacyclin (PGI) by acting in an autocrine fashion on the ET_B subtypes of endothelial cells [24].

In the kidney, ET-1 increases renal vascular resistance and reduces GFR, but also water and Na^+ absorption in the collecting duct mostly via activation of ET_B receptors; ET_B also mediate ET-1 clearance, which may occur in the kidney [25]. Excess ET-1 disrupts the actin cytoskeleton in podocytes via ET_A receptors, favoring proteinuria, glomerulosclerosis, and apoptosis of tubular epithelial cell with ensuing hypoxia and inflammation [26–28].

ALDOSTERONE-MEDIATED IMPAIRMENT OF ENDOTHELIUM-DEPENDENT VASODILATION

Aldosterone is produced by the adrenocortical zona glomerulosa and to a lesser extent locally by vascular endothelial and smooth muscle cells, mesangial cells, and cardiomyocytes [29–35]. By acting via nuclear and cytoplasmic mineralocorticoid receptors [36,37], it regulates vascular tone and vascular, myocardial, and renal structure. Mineralocorticoid receptor activation induces both vasoconstriction and vasodilation. The latter involves

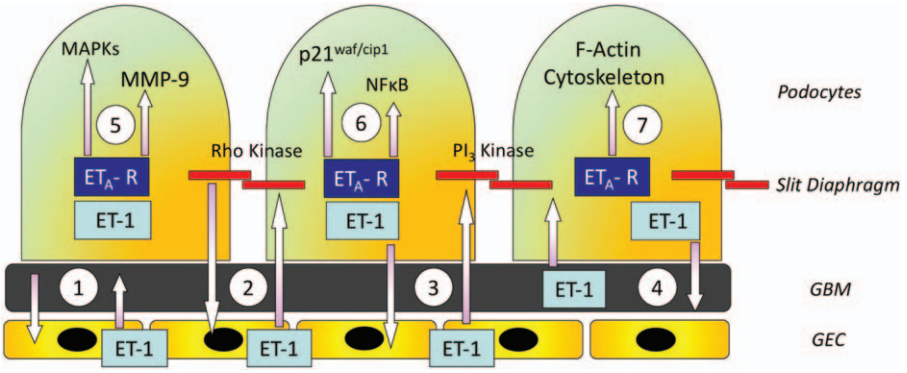

FIGURE 2 Schematic representation of the actions and interactions of endothelin-1 (*blue*) between glomerular capillary basement membrane (*gray*), glomerular endothelial cells (*yellow*), glomerular podocytes (*green/yellowish*), and the slit diaphragm (*red*). Shown are mechanisms (1–7) which implicate endothelial-cell derived endothelin-1 in podocyte and glomerular injury. Endothelin-1 is released from cells on both sides of the glomerular capillary basement membrane, namely glomerular endothelial cells and podocytes. It interacts with different cellular components of the glomerular capillary: endothelin-1 released from glomerular endothelial cells interacts with the glomerular capillary basement membrane (1), with the slit diaphragm (2), or with the podocyte (3). Endothelin-1 release and signaling may also occur in the reverse direction. Accordingly, endothelin-1 released from podocytes may interact with the glomerular capillary basement membrane and vice versa (4). Within the podocyte, endothelin-1 activates endothelin ET_A receptors and promotes glomerular cell injury and sclerosis through MAPKs p38 and p44/p42 pathways (5); moreover, endothelin-1 stimulates growth promoter and cyclin-dependent kinase-inhibitor $p21^{waf/cip1}$, and proinflammatory NF-kappa B (6). Endothelin-1 also causes F-actin cytoskeleton dysruption (7) and thus slit diaphragm dysfunction (*green*) involving activation of the Rho-kinase and PI_3-kinase pathways. Figure adapted from [97].

phosphatidyl-inositol (3,4,5) trisphosphate kinase-dependent activation of nitric oxide synthase (NOS) in the endothelial cells, a rapid effect that is held to be nongenomic, as it is insensitive to inhibitors of gene transcription or protein synthesis [38–41]. Thus, the rapid nongenomic effects are crucially regulated by the endothelial (dys)function: if endothelial function is intact, aldosterone exerts vasodilatation and increase GFR, but when the endothelial function is impaired with blunted endothelial NOS (eNOS) activity, aldosterone causes vasoconstriction, decreased renal plasma flow, and increased renal vascular resistance [39,41]. The vasoconstricting effect is more pronounced in the afferent arteriole than in the efferent arteriole [39].

Recent evidence suggests that some effects of aldosterone involve a cross-talk between the mineralocorticoid receptor and the G-protein-coupled estrogen receptor (GPER) [37,42]. When infused into dogs, in which renal perfusion pressure was clamped to 19 mmHg, aldosterone (14 μg/kg per day) was found to cause hyperfiltration [43], a finding that can explain the hyperfiltration seen in human primary aldosteronism, and the apparent worsening of estimated GFR after cure of the hyperaldosteronism with adrenalectomy [44]. However, the correction of hypervolemia after adrenalectomy may also play a role.

A mineralocorticoid receptor-mediated aldosterone-induced vasoconstriction has been observed in healthy humans in the forearm [38]. However, if coinfused with the inhibitor of eNOS *N*(G) monomethyl-L-arginine, aldosterone (500 μg one time bolus injection) increases renal vascular resistance, with a more pronounced effect on the afferent than the efferent arteriole [41]. Aldosterone regulates Na^+ channels in the endothelium, as it does in the distal tubular epithelial cells and collecting duct [45], and causes endothelial cell swelling, an effect abolished by the epithelial or endothelial Na^+ channel (ENaC) blocker amiloride [46]. It also increases ENaC, a target of aldosterone in distal tubular epithelial cells (see above) and promotes its insertion to the membrane [47] via both genomic and nongenomic effects [45,47–49].

Mice with a specific knock-out of the mineralocorticoid receptor in endothelial cells alone represent an optimal model to investigate the role of this receptor. In this model, a pronounced vasoconstriction was found in coronary but not mesenteric arterioles after exposure to ET-1, despite no change in blood pressure (BP) or renal Na^+ handling [50]. Whether endothelial mineralocorticoid receptor regulates renal hemodynamics remains unclear. However, mice with targeted deletion of mineralocorticoid receptors in vascular smooth muscle, or in endothelial cells, show that only in the former cells the receptor is crucial for the hemodynamic alterations that lead to acute kidney injury induced by subcutaneous cyclosporine administration. Hence, aldosterone, via the mineralocorticoid receptor, promotes microvascular contraction, thereby potentiating the effect of cyclosporine-induced NO deficiency [51].

Furthermore, in a model of acute kidney injury induced by pedicle clamping, the ischemic injury was prevented by antagonizing the mineralocorticoid receptor [52]. Ischemia-induced oxidative stress and cysteine sulfenic acid modification of endothelial cell ET_B receptors causing blunted activation of eNOS were prevented by the mineralocorticoid receptor antagonist [52].

In endothelial cells, aldosterone can also increase the expression of F-actin myofilaments, which, combined to G-actin myofilaments, form a web beneath the membrane that dynamically regulates the lumen caliber [46]. An increased F-to-G-actin myofilament ratio leads to web polymerization with ensuing cell 'stiffening' and decreased NO production [46]. Hence, aldosterone may potently cause renal vasoconstrictor acting via both endothelium stiffening and decreased NO bioactivity. Both Ang II, via AT_1 receptors, and endothelin-1, via ET_A receptors, mediate the effects of aldosterone infusion on BP and end-organ injury [53,54]. T-lymphocytes have also been implicated in aldosterone-mediated effects [55]. Of note, human primary aldosteronism is associated with microalbuminuria, a marker of early endothelial dysfunction, which persists even after correction of hyperfiltration [56], but regresses when primary aldosteronism is cured with adrenalectomy [57].

ENDOTHELIN-1 IN HYPERTENSION AND RENAL DAMAGE

ET-1 contributes to the regulation of vascular tone and BP [58], and to a multitude of other physiological processes, including cell proliferation, endothelial dysfunction, arterial stiffness, cardiac hypertrophy, and tissue fibrosis via epithelial to mesenchymal transition (EMT), and also stimulation of aldosterone secretion, all of which contribute to the development and maintenance of hypertension and its detrimental consequences [59,60]. Vascular production of ET-1 is increased in most salt-sensitive animal models of hypertension [61]. Notably, the pressor effects of ET-1 appear to be, at least in part, dependent on salt, as chronic infusion of a subpressor dose of ET-1 increases BP only when combined with a normal or high-sodium diet [62].

By inducing vasoconstriction, vascular remodeling, and decreasing arterial compliance, ET-1 enhances arterial pulse wave reflection and central pressure augmentation, contributing to arterial stiffening, resulting in a reduced capacity of buffering pressure and pulsatile flow oscillations [63]. Arterial stiffness is independently associated with mortality in ESRD patients and, moreover, worsens rapidly over time in those in hemodialysis [64]. Moreover, infusion of ET-1 in healthy humans which increases plasma levels to those seen in ESRD was found to be associated with significant increases in pulse wave velocity, central systolic pressure, and pulse pressure [65].

In experimental models of hypertension associated with an increase in ET-1, both selective ET_A and nonselective $ET_{A/B}$ receptor antagonists effectively reduce BP [62]. Importantly, selective ET_A receptor blockade prevents endothelial cell dysfunction, vascular hypertrophy, and glomerular sclerosis in salt-dependent genetic hypertension, even when high BP is not fully corrected, suggesting BP-independent beneficial effects of the treatment [60,66–68].

In hypertensive patients, circulating ET-1 levels were found to be increased compared with healthy individuals in some, but not all, reports [69], which can depend on the fact that ET-1 secretion occurs abluminally and the mature peptide has a vey short half-life. According to studies with ET receptor antagonists (ERAs) both selective and nonselective ERAs increase forearm vasodilation and reduce BP more in hypertensive patients than in healthy individuals [69]. Finally, even though several chronic studies have suggested that both ET_A-selective and nonselective $ET_{A/B}$ ERAs can reduce BP [69], no direct head-to-head comparison of the two approaches is available. In CKD plasma ET-1 is increased [70], likely because of augmented production and reduced clearance. Notably, urinary excretion of ET-1, reflecting renal production, increases as renal function declines [70], suggesting that this overactivation of the renal ET system contributes to hypertension and to worsening of renal function.

In hypertensive nondiabetic CKD patients, acute ET_A receptor blockade reduces BP by about 10 mmHg [71], a decrease that is attenuated by concomitant ET_B receptor antagonism [72], suggesting that vasoconstrictor ET_B receptor activity is less important than ET_B vasodilatory and ET-1 clearance function, at least in this condition. In a similar patient population, chronic ET_A receptor antagonism also reduces BP, albeit to a lesser extent [73]. Significantly, in both acute and chronic studies [71,73], the majority of the patients studied were already taking Ang I converting enzyme (ACE) inhibitors, which is important clinically, as after the publication of the RENAAL, IRMA-1, and IRMA-2 studies [74–76], most CKD patients are prescribed RAAS inhibitors, not only for BP control but also for their microalbuminuria-lowering effects. Limited data with nonselective $ET_{A/B}$ antagonism suggest that this approach may also be beneficial in lowering BP in nondiabetic CKD patients [77]. Proteinuria, a manifestation of dysfunction of the glomerular filtration barrier due to podocyte injury, is associated with increased cardiovascular risk. Both acute [71,78] and chronic [73] selective ET_A receptor blockade and nonselective $ET_{A/B}$ antagonism [77] reduce proteinuria in patients with nondiabetic CKD. In the acute studies, these beneficial effects were abolished by concomitant ET_B receptor antagonism [78], suggesting that they involve ET_B receptor activation. Studies in healthy rats suggest that ET_B receptors could play a role for fluid retention and increase vascular permeability, leading to edema, an effect commonly observed after ET_A receptor blockade possibly resulting from ET_B receptor activation [79]. The mechanisms underlying proteinuria and its reduction by AT1 receptor blockers, ACE inhibitors, and ERAs will be discussed in Part II.

Arterial hypertension favors atherosclerotic vascular disease, heart failure and CKD. Hence, there is a relationship between the heart and the kidney, usually referred to, albeit improperly, as the underlying pathophysiology is not clearly known, as 'cardiorenal syndrome'. Endothelial dysfunction occurs in both heart failure and CKD, but whether it is amplified in the cardiorenal syndrome remains to be elucidated [80].

ENDOTHELIAL GLYCOCALYX

The endothelial cell surface is coated with proteoglycans covalently bound to polysaccharide chains synthesized in the endothelial cells [81]. Sulfation of its components, mostly heparan and chondroitin sulfates, makes the glycocalyx a negatively charged gel-like surface. The endothelial glycocalyx provides a passive barrier to water and solute transport, which regulates vascular permeability, and the interaction between endothelial and circulating cells, as leukocyte [81]. In the kidney, the densely packed hyaluronan in the glycocalyx, by anchoring to the glomerular basement membrane, fills the fenestrae of endothelium [82], thus preventing filtration of albumin [83], which is close in size to that of the spaces between the glycosaminoglycan fibers (for a review see [84]).

Factors that cause injury to the endothelium also induce sulfation and deacetylation of heparan sulfate in the glycocalyx, increase the expression of glycoproteins, as selectins and integrins, and activate heparanase in podocytes, resulting in loss of the glycocalyx [85,86]. The increased heparanase activity found in diabetic nephropathy [87,88], alongside the prevention of renal damage in heparanase, [84], supports a protective role of the glycocalix in diabetic nephropathy. Moreover, endothelium-derived factors, as ET-1, can activate heparanase in podocytes, with loss of the

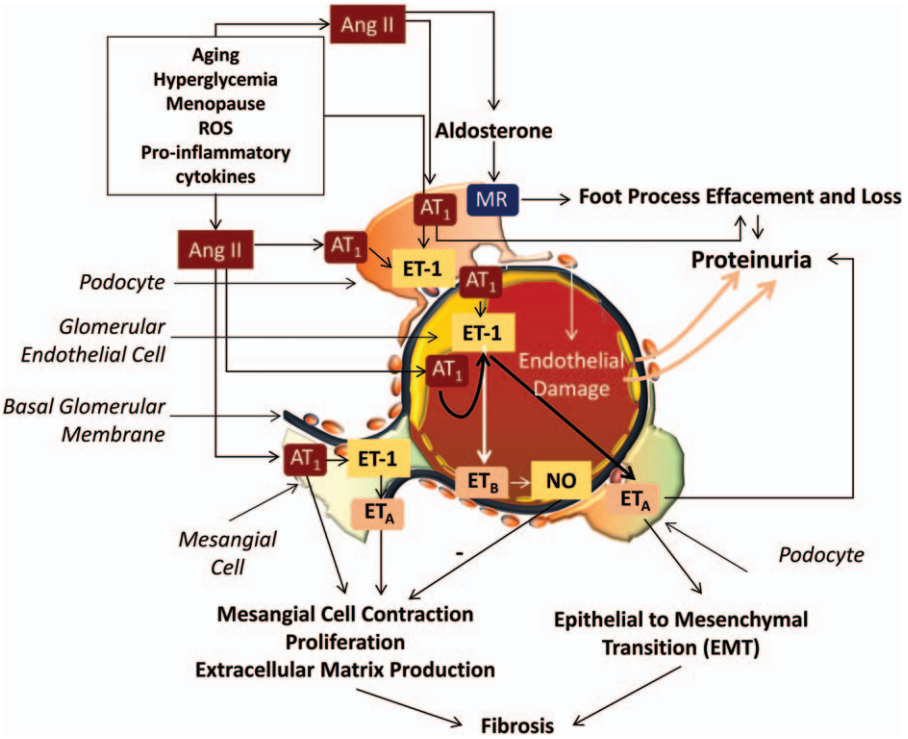

FIGURE 3 Effects of the renin–angiotensin–aldosterone system and the endothelin system in the kidney. Under physiological conditions, angiotensin II maintains glomerular filtration rate by modulating the efferent arteriole tone. It also regulates blood volume by stimulating water and Na^+ absorption in the distal and collecting tubules, both directly and indirectly via aldosterone production. Pathophysiological conditions as aging, hyperglycemia, hypoxia, and inflammation, characterized by release of reactive oxygen species and cytokines, cause excess angiotensin II and endothelin-1 synthesis. In addition, angiotensin II stimulates endothelin-1 synthesis. This translates into constriction of arterioles, both afferent and efferent, with reduced glomerular filtration rate, and also into reduction of nitric oxide bioavailability that causes renin release from juxtaglomerular cells, finally leading to amplification of the renin–angiotensin–aldosterone system activity.

glycocalix. The evidence that podocyte-specific knockout of both ET_A and ET_B receptor subtypes in mice prevented the reduction of heparan sulfate and endothelial glycocalyx thickness and the development of proteinuria supports a harmful role for ET-1 on glycocalix integrity [87].

Excess aldosterone can also affect glycocalyx by enhancing Na^+ binding to glycocalyx, thus saturating its negative surface charges and increasing adhesion of red blood cells, an effect that can be preventd by spironolactone [46]. Hence, aldosterone not only renders the endothelial cells stiffer (see 'ALDOSTERONE-MEDIATED IMPAIRMENT OF ENDOTHELIUM-DEPENDENT VASODILATION' section), but also sticker, finally leading to endothelial damage and vasoconstriction [89].

INTERACTIONS BETWEEN THE RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM, ENDOTHELIN-1, AND NITRIC OXIDE IN THE KIDNEY

The RAAS, ET-1, and NO interact in a complex fashion (Fig. 3) [90]: Ang II was shown to stimulate the expression or release of ET-1 in endothelial cells *in vitro* and to increase ET-1 production in the kidney [22,91]. It also upregulates the expression of the ET_A receptor [92] and the binding of ET-1 to this receptor in endothelial cells *in vitro* [93] and in the kidney *in vivo*. In contrast to Ang II, which whilst constricting all intrarenal vessels has its most prominent effects on the postglomerular vessels, ET-1 causes mainly preglomerular constriction (Fig. 4) [62]. Further actions of the two peptides that occur in parallel in the kidney are mesangial cell contraction, extracellular matrix production, and enhanced tubular Na^+ absorption (ET-1 stimulates sodium excretion via stimulation of the ET_B receptor in proximal tubules, see above), an effect that can be enhanced by the secretagogue actions of both peptides on aldosterone [94].

In transgenic animals overexpressing renin resulting in malignant Ang II-dependent hypertension, ET-1 was shown to promote renal fibrosis by activating EMT [95], a process by which endothelial cells lose their phenotype and acquire that of mesenchymal cells, thus transforming into collagen- producing myofibroblasts [96]. This process leads to microvascular rarefaction and fibrosis, with consequent hypoxia that interferes with endothelial cell repair and regeneration [72].

Glomerular endothelial cell crosstalk with parietal and glomerular epithelial cells (i.e., the epithelial cells that constitute the outer part of the Bowman's capsule and the podocytes, respectively), which release ET-1, Ang II, ROS, cytokines, and other stress-signaling molecules, thus amplifying the injury [97,98]. Figure 3 summarizes this cross-talk, and Figs. 2 and 3 shows the role of ET-1 in this process. An injured endothelium drives innate immunity and inflammation, with complement activation and platelet dysfunction,

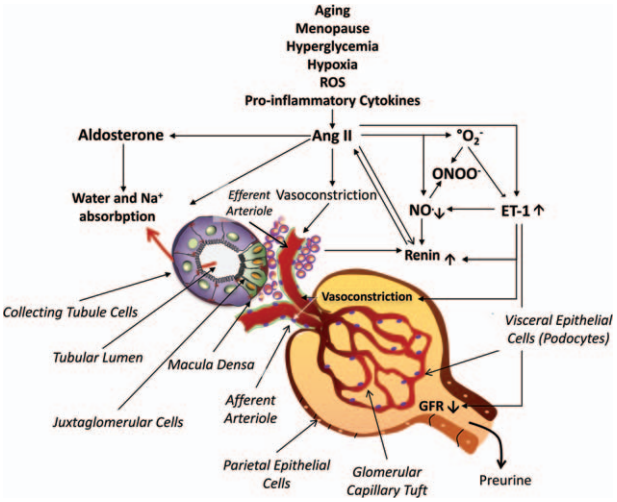

FIGURE 4 Effects of the renin–angiotensin–aldosterone system and the endothelin system in the glomerulus. Under physiological conditions angiotensin II, via AT_1 receptors, regulates the glomerular capillary tone, podocyte function and mesangial cell contraction. Pathophysiological conditions characterized by excess release of reactive oxygen species and proinflammatory cytokines cause excess angiotensin II and endothelin-1 synthesis. Both excess angiotensin II and endothelin-1 induce, via AT_1 and ET_A receptors, respectively, endothelial damage and podocyte injury, which both cause proteinuria, proliferation of the mesangial cells and extracellular matrix production, epithelial to mesenchymal transition finally leading to glomerular sclerosis and fibrosis.

which further enhance the damage [97,99]. Hence, without any safeguard mechanisms, the combined action of Ang II and ET-1 would render the kidney ischemic and fibrotic in a short period of time. However, both systems can also play a protective role by inducing vasodilation and natriuresis and inhibiting fibrosis, either acting directly or through stimulation of NO production. Ang II can raise NO via intrarenal Ang II type 2 (AT_2) receptors, whereas ET-1 does so by activating ET_B receptors. Thus, it is conceivable that both ET-1 and Ang II, or at least its protective breakdown product, Ang 1–7 [100], can also play beneficial effects. Ang 1–7, which is made from Ang I through ACE2 action, can activate eNOS and release of NO via the G-protein-coupled Mas receptor [101]. Notably, Ang II, ET-1, and NO all inhibit renin release, perhaps as a feedback mechanism to reduce angiotensin production [102].

Nonetheless, the importance of these protective mechanisms for different disease conditions remains to be fully established, as their roles vary under pathological conditions, turning a protective into a detrimental mechanism. For example, the AT_2 receptors, commonly regarded as part of the protective arm of the RAAS, can mediate vasoconstriction under conditions of excess ROS production [103]. Similarly, in systemic and pulmonary hypertension ET_B receptors were upregulated in VSMCs of the tunica media, where they can play a pathogenic role [104]. Recent studies have also shown that ET_B receptors mediate tubulo-interstitial fibrosis and its underlying mechanisms, as discussed below, thus further aggravating kidney damage (Fig. 4) [95]. Studies also suggest that eNOS, the enzyme responsible for NO formation, can be stimulated by AT_1 receptor activation, but this effect is probably short-lived. Moreover, a predominant downregulation of eNOS mediated by the AT1 receptor has also been reported [105].

RENAL FIBROSIS IN CHRONIC KIDNEY DISEASE: ROLE OF ENDOTHELIAL FACTORS AND OXIDATIVE STRESS

Renal fibrosis, the final common outcome of practically all renal diseases causing CKD, including hypertensive nephro-angiosclerosis [106], entails a progressive loss of nephrons and their replacement by extracellular matrix ultimately leading to ESRD [106]. Fundamental in this process is EMT that entails the loss of epithelial markers by tubular cells and their acquisition of mesenchymal features, alongside loss of cell contacts, degradation of cell adhesion molecules, onset of migration properties, and finally their transformation into fibroblasts, which synthesize collagen and other extracellular matrix proteins [107]. First described in embryo development and cancer, EMT occurs in the kidney and involves transformation of tubular cells and podocytes into myofibroblasts [108].

ET-1 was found to induce loss of synaptopodin, a podocyte marker, alongside acquisition of the mesenchymal marker α-smooth muscle actin in cultured mouse podocytes [108]. The selective ET_A antagonist sitaxentan prevented these changes [108], thus suggesting a role of this receptor subtype in glomerular EMT. However, more recent work in a transgenic rat model of Ang II-dependent hypertension and in human renal tubular cells, demonstrated a fundamental role of ET-1, acting via ET_B receptors [95]: EMT was prevented *in vivo* by the mixed ET_A/ET_B receptor antagonist bosentan and *in vitro* by the selective ET_B receptor antagonist BQ-788. Figure 5 summarizes the series of events leading to hypertensive nephron-angiosclerosis via EMT and ET_B receptor.

A high level of oxidative stress can also participate in this process: the serum levels of the potent antioxidant ascorbic acid are decreased in CKD patients, likely because of malnutrition and hemodialysis [109,110]. Moreover, serum total antioxidant power is also blunted, supporting the view that high oxidative stress can contribute to blunting NO bioactivity, the main opponent of ET-1, in CKD patients. In keeping with this contention, chronic administration of green tea was shown to restore endothelium vasodilation through ROS scavenging and to protect from hypertension and kidney damage in a rat model of Ang II infusion [111]. Moreover, green tea extracts decreased left ventricular mass and p22phox, an essential component of the ROS-producing NADPH oxidase, in mononuclear cells of CKD patients despite no decrease in BP [112].

OTHER ENDOTHELIAL FACTORS IN RENAL VASCULAR REMODELING

Vascular remodeling and endothelial dysfunction are hallmarks of patients with ESRD. In such patients, arterial remodeling is related inversely to forearm reactive hyperemia and to serum concentrations of markers of endothelial dysfunction [113], suggesting that the latter contributes to vascular changes in ESRD patients [113]. In hypertension, renal endothelial cell dysfunction is characterized by a decreased release of vasodilatory mediators, such as NO, PGI, and EDH, and/or an increase in vasoconstrictive

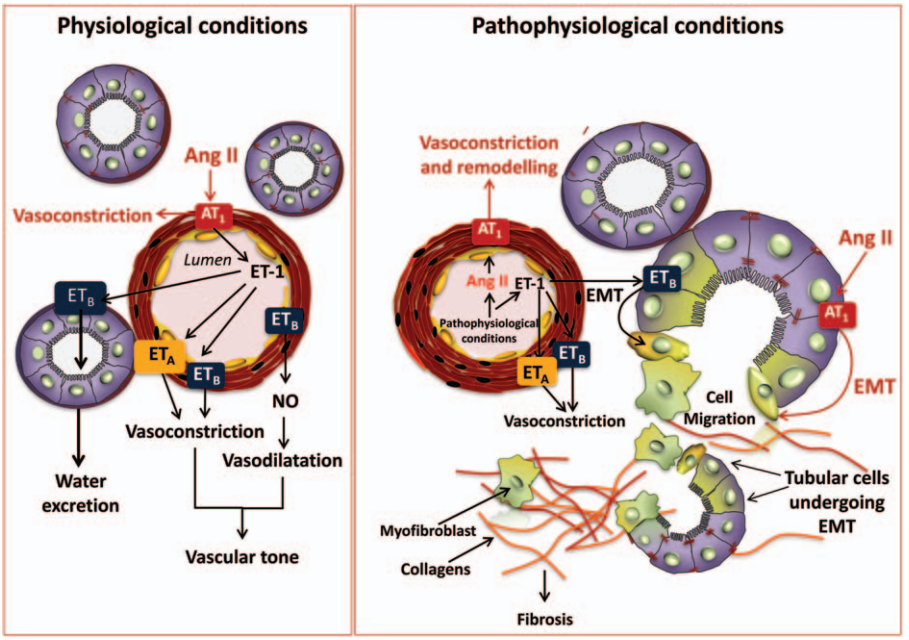

FIGURE 5 Effects of the renin–angiotensin–aldosterone system and the endothelin system in the tubulo-interstitial compartment. Under physiological conditions, both angiotensin II and endothelin-1 maintain the vascular tone: AT1, ET_A, and ET_B receptor subtypes located at the vascular smooth muscle cells mediate vasoconstriction, whereas ET_B receptor subtype located at the endothelial cells, by increasing nitric oxide bioavailability, mediates vasodilatation. ET_B receptor also exerts a crucial role by regulating water excretion at the tubular level. When angiotensin II is abnormally produced, as under conditions characterized by excess mechanical or oxidative stress (e.g., diabetes mellitus, high blood pressure, hypercholesterolemia), AT1 receptors in the vascular smooth muscle cells favor vasoconstriction and vascular remodeling. Excess angiotensin II also stimulates endothelin-1 production that, via ET_A and ET_B located at the vascular smooth muscle cells, potentiates vasoconstriction and, via ET_B receptor subtype, triggers epithelial to mesenchymal transition (EMT), transforming the tubular cells into myofibroblasts that produce collagens, finally leading to tubulo-interstitial fibrosis.

mediators, such as ET-1, Ang II, and thromboxane A_2 (TXA), as discussed above [19]. This may affect matrix metalloproteinases and their inhibitors resulting in changes of the extracellular matrix composition, leading to renal vascular remodeling [19]. Therapeutic interventions can modulate the synthesis and release of both endothelium-derived relaxing and contracting factors, thus influencing the vascular remodeling process [114].

Exogenous factors, such as high salt intake, but also vascular inflammation, further promote endothelial dysfunction in the kidney [115,116], causing increased expression of adhesion molecules, activation of immune cells, cytokine production, and increased oxidative stress [115–117]. Infiltration of immune cells in various organs such as blood vessels, kidney, and perivascular adipose tissue is an important component of the inflammatory process leading to cardiovascular damage and hypertension [117]. Evidence has accumulated on the participation of T lymphocytes in hypertension, particularly through effects on the kidney [117]. Once activated, Th1 cells may contribute to BP elevation by affecting the kidney and vascular remodeling of blood vessels directly via the effects of the cytokines produced [117]. By contrast, T-regulatory cells might protect from BP elevation by acting on similar targets [117]. T-regulatory cells and Th1 lymphocyte subtypes also have also opposite effects on endothelial function [117] and on microvascular remodeling [118]. An imbalance of T-cell subsets was observed in Ang II-infused hypertensive rats with kidney injury [119]. Finally, other possible factors linking endothelial factors and vascular morphology include endothelial progenitor cells, which are involved in vascular remodeling and repair and are regarded as another marker of arterial injury in patients with CKD [120].

CONCLUSION

Compelling evidence indicates that the endothelium plays a fundamental role in preserving vascular health in practically all organs, and the kidney is no exception to this rule. Given the key roles of the glomerular and tubular microvasculature in maintaining renal function, preserving and restoring endothelial function and preventing endothelial dysfunction seem to be fundamental for preserving GFR and preventing CKD. The novel knowledge generated on the biology of the endothelium and its derived factors has made substantial contributions to understanding the mechanisms of kidney damage in cardiovascular and metabolic diseases. In the companion article (Part II), we specifically examine endothelial dysfunction in diabetes, preeclampsia, kidney transplantation, and how to improve the treatment and prevention of kidney disease.

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Conflicts of interest

There are no conflicts of interest.

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