The Endothelin System and Its Antagonism in Chronic Kidney Disease

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The incidence of chronic kidney disease (CKD) is increasing worldwide. Cardiovascular disease (CVD) is strongly associated with CKD and constitutes one of its major causes of morbidity and mortality. Treatments that slow the progression of CKD and improve the cardiovascular risk profile of patients with CKD are needed. The endothelins (ET) are a family of related peptides, of which ET-1 is the most powerful endogenous vasoconstrictor and the predominant isoform in the cardiovascular and renal systems. The ET system has been widely implicated in both CVD and CKD. ET-1 contributes to the pathogenesis and maintenance of hypertension and arterial stiffness and more novel cardiovascular risk factors such as oxidative stress and inflammation. Through these, ET also contributes to endothelial dysfunction and atherosclerosis. By reversal of these effects, ET antagonists may reduce cardiovascular risk. In particular relation to the kidney, antagonism of the ET system may be of benefit in improving renal hemodynamics and reducing proteinuria. ET likely also is involved in progression of renal disease, and data are emerging to suggest a synergistic role for ET receptor antagonists with angiotensin-converting enzyme inhibitors in slowing CKD progression.

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Chronic kidney disease (CKD) is common. A US population study suggested that >10% of the general adult population have an indicator of kidney damage: proteinuria, hematuria, and/or reduced GFR (1). Despite our best current treatments, progression to ESRD remains a major clinical and financial problem, and currently >1 million patients worldwide are on dialysis, with the number continuing to increase yearly. Medicare expenditure on dialysis totaled $14.8 billion in 2003 (2).

It is now widely recognized that cardiovascular disease (CVD) is strongly associated with CKD (1,3) and constitutes one of its major causes of morbidity and mortality (1). Indeed, CKD has emerged as an important and powerful independent risk factor for CVD (1). As GFR declines, the risk for CVD increases, and patients with CKD that do not require dialysis are more likely to die from CVD than to develop ESRD (1). Furthermore, not only are individuals with CKD at increased risk for cardiovascular events, but also their outcome is worse than in those without CKD (4). Although the prevalence of traditional risk factors (*e.g.*, diabetes, hypertension, dyslipidemia) in the CKD population is high, CVD events remain disproportionate to the underlying risk factor profile (1). Therefore, "nontraditional" risk factors, such as endothelial dysfunction, arterial stiffness, oxidative stress, and acute-phase inflammation, which may contribute to this excessive uremic cardiovascular risk, have become a major focus of interest.

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There is an important unmet need for treatments that not only slow the rate of progression of renal impairment, delaying the onset of dialysis in CKD, but also improve the cardiovascular risk profile in these patients. Blockade of the endothelin (ET) system has emerged as one potential strategy. The ET system has been widely implicated in renal disease, including acute renal failure (5). However, the focus of this review is to examine our current understanding of the role that the ET system plays in CKD and whether inhibition of its actions might slow the progression of CKD and reduce the burden of CVD with which it is associated.

Biology of the ET System

First described by Yanagisawa *et al.* in 1988 (6), the ET system is a family of 21 amino acid peptides with powerful vasoconstrictor and pressor properties. Three different isopeptides, ET-1, ET-2, and ET-3, are known, each with distinct gene and tissue distributions (6–8). Of the three peptides, ET-1 is the major endothelial isoform and, in the human kidney, the only one that has been so far shown to be expressed at the protein level (9). Its main site of vascular production is the endothelial cell, but it is also produced by other cell types, including vascular smooth muscle cells and epicardial cells (10). Within the kidney, it is produced by glomerular epithelial and mesangial cells and renal tubular and medullary collecting duct cells (5). Furthermore, the renal medulla is not only an important site of ET-1 generation but also contains among the highest concentrations of immunoreactive ET-1 of any organ (11).

The gene product is the 212 amino acid prepro-ET-1, and regulation of ET synthesis occurs at the level of gene transcription. Enhanced generation occurs with a wide range of stimuli

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(12,13). Those pertinent to CKD include other vasoactive hormones, such as angiotensin (AngII) and vasopressin (AVP), the cytokine IL-1, oxidized LDL, reduced extracellular pH, and cyclosporin A. In contrast, prostacyclin, nitric oxide (NO), and the natriuretic peptides all inhibit gene transcription. Prepro-ET-1 is cleaved to big ET-1 (38 amino acids), which is largely biologically inactive (14). ET-converting enzyme then catalyzes generation of the biologically active ET-1 and C-terminal fragment from big ET-1. Once synthesized, the secretion of mature ET-1 from endothelial cells is largely abluminal (15), toward the adjacent vascular smooth muscle, suggesting an autocrine or paracrine mechanism of action.

ET-1 Action in the Vascular System

ET-1 acts by binding to two distinct receptors, the ET_A and ET_B receptors (16,17) (Figures 1 and 2). Within blood vessels, ET_A receptors are found on smooth muscle cells, and their activation results in vasoconstriction. ET_B receptors are also found on vascular smooth muscle cells (18), where they can mediate vasoconstriction, but are predominantly found on the vascular endothelium, where their activation results in vasodilation *via* prostacyclin and NO (19).

In addition to its vasodilatory function, the ET_B receptor also acts as a clearance receptor for circulating ET-1. The half-life of ET-1 in the healthy circulation is approximately 1 min (20), with removal through receptor- and non–receptor-mediated mechanisms. ET-1 binds to ET_B receptors, with subsequent ligand–receptor complex internalization and intracellular degradation accounting for the majority of clearance, particularly in the pulmonary circulation (21), although the splanchnic and renal circulations also contribute (12). Therefore, reductions in ET_B numbers, or ET_B receptor blockade, may reduce ET-1 clearance, increasing plasma concentrations without altering production. Importantly, because most ET-1 is released abluminally, plasma concentrations of ET-1 do not accurately reflect ET-1 production.

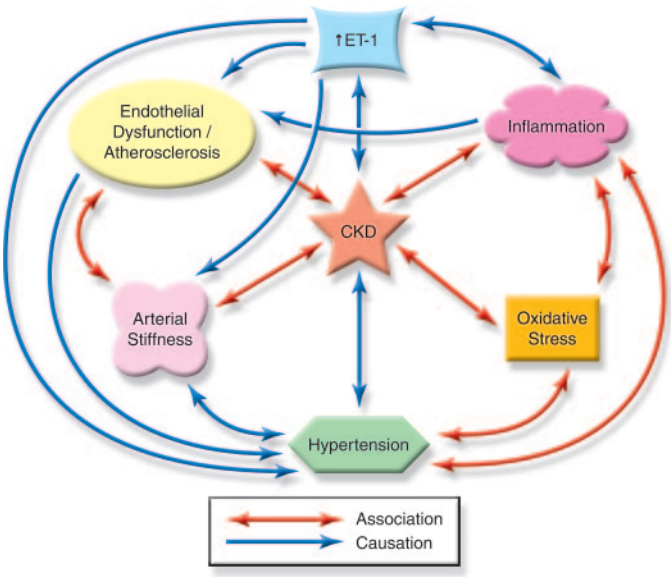

Figure 1. The role of endothelin-1 in chronic kidney disease (CKD) and cardiovascular disease. ET, endothelin. Illustration by Josh Gramling—Gramling Medical Illustration.

ET-1 Action in the Renal System

ET receptors are widely distributed within the human kidney, with the ET_A subtype localized to vascular smooth muscle, notably in the glomeruli, vasa recta, and arcuate arteries, whereas ET_B receptors are more numerous (ET_B to ET_A ratio 2:1) and more widespread, with a high concentration in the collecting system (9,22). With respect to the renal system, ET-1 has a role in the paracrine/autocrine regulation of renal and intrarenal blood flow, glomerular hemodynamics, sodium and water homeostasis (23), and acid-base balance (24) (Figure 1). It is also clear that ET-1 has many other functions within the kidney (25). Evidence exists for renal and vascular ET-1 acting as two independent systems (26). After systemic infusion of radiolabeled ET-1, labeled compound makes up <% of total urinary ET-1 (27). Therefore, neither glomerular filtration nor tubular secretion of plasma ET-1 accounts for urinary ET-1, which is assumed therefore to be primarily of renal origin. Urinary excretion of ET-1 is therefore thought to reflect renal ET-1 production.

Defining the Role of ET in Physiology

ET-1 is a potent vasoconstrictor *in vitro* and pressor in whole animals (28). With respect to the kidney, exogenous ET-1 causes renal vasoconstriction (29). Indeed, the renal vasculature is more sensitive to the vasoconstricting effects of ET-1 than other vascular beds (30). Although exogenous ET-1 reduces total renal blood flow (RBF), a regional difference has been observed, with cortical vasoconstriction (31–33) and NO-dependent medullary vasodilation (31). Exogenous ET-1 has also been shown to cause constriction of afferent and efferent arterioles, with a greater effect on the former (34), and reduce filtration coefficient by mesangial cell contraction (25). In humans, a similar vasoconstrictor (14) and pressor response has been demonstrated (35), as well as renal vasoconstriction, a fall in total RBF, and a consequent reduction in GFR (36). As yet, there are no studies of the effects of ET-1 on intrarenal distribution of blood flow in humans.

With respect to renal tubular functions, there is now a substantial body of evidence supporting a role for ET-1 in the regulation of volume homeostasis. ET-1 inhibits the AVP-stimulated retention of water in inner medullary collecting duct cells *in vitro* (37), and extracellular sodium concentrations may regulate inner medullary collecting duct ET-1 production (23,38). In addition, ET-1 seems to have a natriuretic role, at least in animals. ET-1, acting *via* ET_B and NO, can inhibit chloride transport in the medullary thick ascending limb of Henle, thereby promoting natriuresis (39,40). Picomolar ET-1, binding to ET_B receptors, activates amiloride-sensitive sodium channels in distal tubular cells *in vitro*, although higher, nanomolar doses inhibit this channel by a non–ET_B receptor–dependent mechanism (41). This has been supported by *in vivo* experiments in rats demonstrating natriuresis as a result of reduced sodium transport in the proximal and distal nephron segments in response to low-dose exogenous ET-1, with higher

Actions of ET-1 in the systemic vasculature - animal studies

ET_A receptor	ET_B receptor
Vasoconstriction (28, 59) Increased arterial stiffness (94, 95) Endothelial dysfunction (78-80) Inflammation (102, 104) Atherosclerosis (78, 82) Cardiac hypertrophy (159)	Vasoconstriction (28, 59) Endothelium-dependent vasodilation (59) ET-1 clearance (158)

Actions of ET-1 in the kidney - animal studies

ET_A receptor	ET_B receptor
Renal vasoconstriction (46, 47) Cortical vasoconstriction (31, 32, 46) Afferent arteriolar constriction (34) Efferent arteriolar dilation (34) Mesangial cell contraction (25) Mesangial cell proliferation (25) Extracellular matrix accumulation (160) Interstitial fibrosis (136)	Renal vasodilation (58) Medullary vasodilation (31, 32, 46) Afferent arteriolar constriction (34) Efferent arteriolar dilation (34) Natriuresis (39, 40, 55, 58)

Receptor uncertain

Podocyte de-differentiation (125)
Diuresis (68)
Acid-base balance (24)

Animal models of CKD improved by ET receptor antagonism

Streptozocin-induced diabetes mellitus (112)
Renal mass reduction (150)
Proliferative glomerulonephritis (161)
Lupus nephritis (162)
Hypertensive nephrosclerosis (163)
Chronic CyA administration (114)
Hypokalemic nephropathy (164)

ET receptor antagonist studies in CKD patients

Effect	*ET receptor blockade*	*Dosing*
↓forearm blood flow	ET_A	Acute (63)
↓BP	ET_A, $ET_{A/B}$	Acute (52)
↓RVR, ↓proteinuria	ET_A	Acute (52)
↓proteinuria	ET_A	Chronic (116, 117)

Figure 2. Actions of endothelin-1. Except where indicated, most of the data are drawn from studies in animals. This table shows the receptor responsible for each action but, particularly in the case of ET_A receptor–mediated actions, does not exclude a small contribution from the ET_B receptor. CyA, cyclosporine; RVR, renal vascular resistance. Illustration by Josh Gramling—Gramling Medical Illustration.

doses resulting in sodium retention as a result of glomerular vasoconstriction (42).

Agonist studies may not adequately represent the effects of a hormone, the actions of which are largely autocrine/paracrine, and inhibition of the production or actions of ET-1 may better define its physiologic and pathologic effects. In this respect, ET receptor antagonists have proved to be useful tools in defining the role of ET in health and disease. ET receptor antagonists are classified as ET_A selective (*e.g.*, the intravenous antagonist BQ-123) or ET_B selective (*e.g.*, the intravenous antagonist BQ-788), depending on their relative affinity for a receptor subtype, or nonselective (*e.g.*, the oral drug bosentan) (12). It should be noted, however, that the distinction between selective and nonselective antagonists is not pharmacologically well defined. The so-called "nonselective" antagonists are still selective for the ET_A receptor, but the ratio of ET_A to ET_B affinity is generally 10-

to 100-fold selective for ET_A over ET_B, compared with 1000-fold or more for recent ET_A selective agents (12).

With respect to systemic hemodynamics in healthy humans, selective ET_A receptor antagonism is associated with vasodilation (43) and a reduction in BP (44), and selective ET_B receptor antagonism is associated with vasoconstriction (43) and a pressor response (45). This suggests that endogenous ET-1 contributes to the maintenance of vascular tone and BP *via* the ET_A receptor, and the balance of ET_B receptor function favors activation of the endothelial over the vascular smooth muscle ET_B receptor.

In the kidney, animal studies have suggested that both exogenous and endogenous ET-1–mediated reductions in total RBF are mediated *via* the ET_A receptor (46,47). Antagonist studies describe cortical vasoconstriction as ET_A receptor mediated and medullary vasodilation as ET_B receptor mediated (31,32,46). Furthermore, *in vitro* studies have shown that combined $ET_{A/B}$ receptor antagonism is required to abrogate fully the vasoconstricting effects of exogenous ET-1 on the afferent arteriole, suggesting that both ET_A and vascular smooth muscle cell ET_B receptors are involved. At the efferent arteriole, the effect of ET-1 is blocked by ET_A receptor antagonism alone and enhanced by ET_B receptor blockade, suggesting that ET-1 can modulate efferent arteriolar tone *via* the ET_A receptor and that the balance of ET_B receptor effects here is to produce vasodilation (34). The situation is less clear in healthy humans, where there are few studies. One study has demonstrated an increase in RBF after nonselective ET receptor blockade (48). Most, however, do not demonstrate an effect of selective ET_A receptor blockade (49–53) or combined $ET_{A/B}$ receptor blockade (52) on basal renal hemodynamics, suggesting that ET-1 acting *via* the ET_A receptor does not contribute to the maintenance of renal vascular tone in health. Selective and unopposed ET_B receptor antagonism, however, can produce profound renal vasoconstriction, suggesting that ET-1–mediated tonic renal vasodilation *via* the ET_B receptor is important (52).

Studies have suggested a natriuretic role for the tubular ET_B receptor that is linked to NO generation. A potent inhibitory action of NO on tubular sodium reabsorption is well described (54). A rat model deficient in renal ET_B receptors displays a salt-sensitive hypertension, with restoration of normal BP by amiloride, suggesting that the ET_B receptor regulates sodium excretion at the epithelial sodium channel in collecting duct cells (55), and ET_B antagonist-treated rats develop a sodium-dependent hypertension (56). In addition, in the face of acute ET_B receptor blockade, pressure-natriuresis curves are shifted to the right such that a greater renal perfusion pressure is needed to excrete the same amount of sodium (57). Finally, administration of exogenous low-dose ET-1 to dogs in the presence of high-grade selective ET_A receptor blockade results in renal vasodilation and natriuresis, presumably by unmasking an ET_B receptor–mediated effect (58). Dissecting the different actions of the intrarenal ET system, however, has proved difficult, in part from an inability to discriminate between effects of ET-1 *in vivo* on the nephron and vasculature. To date, ET-1–associated natriuresis and diuresis have not been demonstrated in humans.

Defining the Role of ET in Pathophysiology

Hypertension

Initial evidence of a pressor action of ET-1 led to the suggestion that ET-1 might be implicated in hypertension (6). Production of vascular ET-1 is increased in some (*e.g.*, the Dahl salt-sensitive and the stroke-prone spontaneously hypertensive rat) but not all animal models of hypertension (59). Models in which ET-1 production is increased (mostly but not exclusively salt-dependent types) are associated with increased vascular growth and a response to both selective and nonselective ET receptor antagonism that comprises not only a modest reduction in BP but also a marked regression of vascular growth (59).

In humans, ET-1 message and protein are increased in the vascular smooth muscle cells of hypertensive patients (60). Elevated plasma ET-1 concentrations, however, are not a consistent finding (59). High concentrations would seem, mostly, to be a feature of severe hypertension or indicative of the presence of complications or coexisting disease. Some (61) but not all (62) local studies with ET receptor antagonists have suggested increased vascular ET system activity in patients with hypertension compared with normotensive control subjects and a greater forearm vascular response to nonselective receptor antagonism compared with selective ET_A antagonism, consistent with an increased importance of vascular smooth muscle vasoconstrictor ET_B receptors in hypertension. In CKD, local administration of BQ-123 increases forearm blood flow (63). Systemic administration of BQ-123 ($\pm$BQ-788) to hypertensive patients with CKD showed that selective ET_A receptor blockade produces a substantial reduction in BP (approximately 10 mmHg), whereas nonselective $ET_{A/B}$ receptor antagonism lowered BP to a lesser extent. In both cases, the reduction in BP was much greater than that in healthy control subjects (52), supporting an upregulation of the ET-1 system in CKD-associated hypertension. Only one major study has examined the longer term antihypertensive effects of ET receptor antagonism in humans. Bosentan, an orally available, nonselective ET receptor antagonist, reduced BP in patients with essential hypertension as much as did enalapril 20 mg (64). Importantly, this reduction was achieved without activation of the sympathetic nervous or renin-angiotensin system (RAS).

Altered intrarenal ET-1 production may contribute to hypertension (65,66). Spontaneously hypertensive rats have reduced medullary ET-1 levels after the development of hypertension (67). More recently, Kohan *et al.* (66) successfully created an elegant tissue-specific knockout of the renal ET system. Mice that lack collecting duct expression of the ET-1 gene have reduced urinary ET-1. These animals are hypertensive and have an impaired ability to excrete a sodium load. It is interesting that these knockout mice excrete acute water loads less well than do wild-type mice and have a heightened physiologic response to AVP, consistent with an intrarenal role for ET-1 in blunting the response to AVP (68). Whereas plasma ET-1 concentrations are normal, urinary ET-1 excretion is reduced in hypertensive patients compared with healthy control subjects, suggesting that either renal ET-1 synthesis is reduced or breakdown is enhanced (69,70). Therefore, renal ET-1 production or handling may be altered in hypertension, leading to inappro-

priate sodium and water retention and aiding the development and/or maintenance of hypertension.

Renal function may also influence the relationship between ET-1 and hypertension. First, as renal function declines, plasma ET-1 levels increase (70,71). The effects of exogenous ET-1 on the renal vasculature are to cause vasoconstriction, activating the RAS and causing salt and water retention, both of which have the potential to raise BP. It remains to be seen whether the rise in ET-1 concentrations that is seen in CKD is due to biologically active or simply immunologically competent peptide, but infusion of exogenous ET-1 to bilaterally nephrectomized rats results in an increased plasma half-life of ET-1 and a prolonged rise in BP compared with sham-operated rats (72), consistent with the idea that elevated plasma ET-1 concentrations in CKD may cause hypertension. Second, there is an upregulation of renal ET-1 in CKD (73), as reflected by increased urinary ET-1 excretion (70). Third, there is a suggestion from an experimental model of nephritis associated with mesangial proliferation that the renal vasculature in this disease may be more sensitive to the vasoconstrictor effects of ET-1 than in normal kidneys (74). Therefore, an amplification of the renal vasoconstrictor effects of ET-1, promoting hypertension, could be envisaged in CKD.

Studies of ET_B receptor knockout animals suggest the ET_B receptors are important in protecting against hypertension. These animals exhibit a sodium-dependent hypertension that is attributed to an absence of tonic inhibition of the epithelial sodium channel in the distal nephron (55). It is interesting that ET_B receptor–deficient mice show renal injury, an impaired ability to excrete a sodium load, and hypertension that persists when they are cross-transplanted with wild-type kidneys, suggesting that it may be not only renal but also extrarenal ET_B receptors that play a protective role against hypertension (75).

Endothelial Dysfunction and Atherosclerosis

The endothelium is a crucial regulator of vascular tone (76), and its function is impaired, both in hypertension and in groups who are at risk for hypertension, with a shift toward reduced vasodilation, associated with a proinflammatory and prothrombotic state. Endothelial dysfunction (ED) is also a widely recognized feature of CKD (76), is recognized to be a key early determinant in the progression to atherosclerosis, and is now well established to be independently associated with increased cardiovascular risk (77). Mechanisms that participate in ED include reduced NO generation, oxidative stress, and upregulation of inflammatory mediators (76). Animal models of ED across a number of animal species have shown that antagonism of the ET system, predominantly with selective ET_A receptor antagonists, improves NO-mediated endothelial function (78–80), suggesting that ET-1, acting *via* ET_A receptors, is involved in the pathogenesis of ED. Treatment with selective ET_A receptor antagonism also improves endothelial function in the coronary vessels of patients with atherosclerosis, again suggesting that ET-1, acting *via* ET_A receptors, is involved in the pathogenesis of ED in these patients (81).

The ET system is also implicated in the development of atherosclerosis. In smooth muscle cells and foamy macrophages in atherosclerotic models, both ET_A and ET_B receptors are highly expressed (82). Increased expression of ET-1 and ET-converting enzyme is seen in human arteries at different stages of atherosclerosis (60,83), and high levels of ET-1 have been found in human atherosclerotic lesions (60,83–85). Furthermore, plasma ET-1 concentrations correlate positively with the degree of atherosclerosis present (84). Importantly, not only is restoration of the impaired activity of the NO system and, hence, improvement in endothelial function seen after ET receptor antagonism in a range of animal models of atherosclerosis (78,79,82), but so too is a reversal of atherosclerotic lesion development. Therefore, ET antagonists reduce the activity of the ET system, increase NO bioavailability, improve endothelial function, and slow the progression of atherosclerosis. To date, there are no therapeutic studies on endothelial function or atheroma progression with ET antagonism in patients with CKD.

Arterial Stiffness

Arterial stiffness is an important independent predictor of all-cause and cardiovascular mortality in patients with ESRD (86). Moreover, a therapeutic trial in patients with ESRD by Guerin *et al.* (87) showed that after BP reduction, cardiovascular survival was observed mainly in patients who also displayed a reduction in arterial stiffness (87). Epidemiologic studies indicate that there is an increased cardiovascular risk early on in CKD, but there are as yet few data that show how early arterial stiffness develops (88). Increased arterial stiffness results in a selective elevation of pulse pressure, causing deleterious consequences for the heart. Through an elevation of central systolic BP, arterial stiffness enhances left ventricular load, favoring development of cardiac hypertrophy, and through reduction of central diastolic BP, it decreases coronary perfusion pressure, contributing to myocardial ischemia (89).

Arterial stiffness is linked to ED (89), and the two conditions commonly coexist in patients who are at increased risk for CVD. A number of interventions that reduce arterial stiffness also improve endothelial function (89). To date, few studies have addressed the relationship between these two markers of CVD after treatment and none in patients with CKD. However, there now is evidence from both animal and human studies that the endothelium is an important regulator of arterial stiffness. Basal endogenous NO generation decreases arterial stiffness in animals (90) and humans (91–93). By contrast, ET-1, at concentrations similar to those observed in the plasma of patients with CKD, caused an increase in arterial stiffness that can be blocked by concomitant administration of an ET_A receptor antagonist (94). Furthermore, endogenous ET-1 was shown recently to increase arterial stiffness (95). Therefore, in ED, where NO is downregulated and ET-1 is upregulated, the balance will likely shift in favor of increased arterial stiffness. Clinical studies of the effects of ET receptor antagonism on arterial stiffness in CKD will clearly be of great interest.

Oxidative Stress and Inflammation

Oxidative stress and inflammation are well documented in ESRD (96,97). Indeed, they are very common even with mild

renal insufficiency (98). Oxidative stress is characterized by an imbalance between exposure to free radicals, principally derived from oxygen, and antioxidant defenses. As in ED, there is loss of NO availability in states of oxidative stress, and the close relationship among increased oxidative stress, reduced NO availability, and subsequent cardiovascular events is well established (99). In addition, there is now mounting evidence, although scarce human data, supporting the hypothesis that at least some of the injurious effects of ET-1 on the vasculature are mediated *via* an increase in oxidative stress and that ET system blockade may be of use in reducing this (100,101). Indeed, in DOCA-salt hypertension, the ET-1–promoted production of reactive oxygen species, the principal mediators of oxidative stress, is ET_A receptor mediated, and selective ET_A receptor blockade normalizes the ED found in this model (102), independent of changes in BP. Data in CKD are scarce at present, and it remains unclear whether oxidative stress is a cause or a consequence of renal insufficiency.

Reactive oxygen species likely promote the development of atherosclerosis through a number of mechanisms (103), including ED, increased production of proinflammatory cytokines such as IL-6, and acute-phase proteins such as C-reactive protein (CRP) (104). IL-6 and CRP are both independent predictors of cardiovascular events and mortality (105). Recent evidence suggests that a reduction in kidney function *per se* may be associated with an inflammatory response in both mild (106) and advanced (107) kidney disease, and a number of studies have shown that CRP predicts all-cause and cardiovascular mortality in dialysis patients (108). In addition to being an important prognostic marker for CVD, CRP may contribute to the atherosclerotic process mainly through the impairment of endothelial function (109,110). Furthermore, CRP has been shown to decrease the activity of the NO system (111) and to potentiate the release of ET-1 (104). ET receptor antagonism has been shown to attenuate the proatherogenic effects of CRP *in vitro* (104), consistent with anti-inflammatory and antiatherogenic actions.

Defining the Role of ET in Renal Pathophysiology

CKD and Renal Hemodynamics

There are only few studies in animal models of CKD. Nevertheless, these show that ET receptor antagonism improves RBF and preserves GFR. Nonselective $ET_{A/B}$ receptor antagonism with bosentan can prevent the renal vasoconstriction that is seen in the early phases of streptozocin-induced diabetes (112), and selective ET_A receptor antagonism can preserve GFR and RBF during acute (113) and chronic cyclosporin A administration (114). In addition, in the Dahl salt-sensitive hypertensive rat, where a reduced RBF and GFR are observed after a high-salt diet, systemic $ET_{A/B}$ receptor antagonism tended to increase and intrarenal interstitial infusion significantly increased RBF and GFR (115).

In patients with CKD, selective ET_A receptor antagonism produces an increase in RBF and a decrease in renovascular resistance (52), suggesting that ET-1 acting *via* ET_A receptors is involved in the increased renovascular tone. These changes are accompanied by a fall in effective filtration fraction (EFF), suggesting that ET-1, acting *via* ET_A receptors, exerts a preferential efferent arteriolar vasoconstrictive effect, raising the possibility that ET-1 promotes hyperfiltration with its consequent potential for renal injury. The renal hemodynamic effects of selective ET_A receptor antagonism can be abolished by concomitant administration of an ET_B receptor antagonist, and, as in health, selective blockade of the ET_B receptor produced renal vasoconstriction (52). Notably, in these studies, selective ET_B receptor antagonism increased renal vascular resistance by twice as much (approximately 20 to 30%) as systemic vascular resistance (approximately 10 to 15%), suggesting that tonic ET_B receptor–mediated renal vasodilation plays a key role in opposing renal vasoconstriction. This is likely to be of particular importance in CKD, where baseline renal vascular resistance is high. The renal hemodynamic changes in these studies are consistent with other work in patients with CKD, in whom nonselective ET receptor blockade reduces EFF while maintaining GFR (56), and in patients who have diabetes with albuminuria, in whom selective ET_A receptor antagonism reduced both BP and urinary protein excretion (116,117).

Proteinuria

Significant proteinuria has emerged as a powerful predictor of renal disease progression (118), and proteinuria reduction is an important strategy to retard or prevent renal functional loss (118,119). In addition, proteinuria is no longer simply a renal risk factor. Alongside the concept of CKD as a global vascular disease state is emerging the global cardiovascular risk that is associated with proteinuria. Albuminuria is incrementally associated with increased cardiovascular risk in both individuals with preexisting risk (*e.g.*, hypertensive patients) (120) and individuals with no known risk factor (121). This is true even in the presence of normal renal function (122). Furthermore, at least in hypertension, reduction of albuminuria confers cardiovascular protection (120).

Upregulation of the renal ET system exacerbates proteinuria. Through its hemodynamic effects, ET-1 causes glomerular capillary hypertension, an increase in glomerular permeability, and excessive protein filtration (123). A reduction in microalbuminuria in patients with diabetes was shown recently after chronic selective ET_A receptor blockade (116,117). Furthermore, the reductions in EFF that were observed after acute selective ET_A receptor antagonism in patients with CKD were accompanied by a reduction in proteinuria (52), suggesting that one mechanism for the antiproteinuric effect of ET_A receptor antagonism in CKD may relate to alterations in glomerular hemodynamics with, potentially, a fall in glomerular capillary perfusion pressure. This would produce a situation analogous to that seen with angiotensin-converting enzyme (ACE) inhibitors (118), in which case ET antagonists might be expected to be renoprotective and improve long-term renal outcome.

The development of proteinuria is also associated with damage to the renal podocyte (124), the highly specialized glomerular epithelial cell that helps to maintain an intact filtration barrier under normal conditions. Recent *in vitro* studies suggested that podocytes undergo phenotypic changes that resem-

ble de-differentiation as a result of exposure to exuberant amounts of protein (125). In parallel with these changes, there was increased ET-1 production by the podocyte, which was dependent, at least partly, on the cytoskeletal rearrangements that were brought about by excess protein exposure. In the same model, administration of exogenous ET-1 brought about similar podocyte cytoskeletal changes. Therefore, the authors concluded that podocyte-derived ET-1 acting in an autocrine and paracrine manner promotes further podocyte ultrastructural degeneration and hence its own production, both of these contributing to a further breakdown in the glomerular filtration barrier. These data are in line with *in vivo* evidence in a murine model of protein overload that displays increased renal ET-1 production alongside the development of podocyte structural damage (126). Whether damage to the podocyte is the primary event that leads to subsequent proteinuria or *vice versa* remains unclear. Nevertheless, it is not unreasonable to envisage a series of events with initial ET-1–mediated glomerular hypertension exposing podocytes to unusually large amounts of protein and so leading to their de-differentiation and production of ET-1. This podocyte-derived ET-1 then could exacerbate glomerular hypertension and lead to further podocyte de-differentiation, thereby setting up a vicious cycle.

Clinical studies are supportive of a role for the ET system in proteinuric nephropathies. Patients with chronic glomerulonephritis and proteinuria displayed a rise in renal ET-1 and tubular ET_B receptor expression that increased with higher degrees of proteinuria (127), and patients who were exposed to selective ET_A receptor antagonism both in the acute (52) and the chronic (117) setting showed a significant reduction in proteinuria.

CKD Progression

Excess protein filtration at the glomerulus leads to increased tubular reabsorption. This can activate tubular-dependent pathways of interstitial inflammation and fibrosis, with progressive renal scarring (128). Studies suggest a link between upregulation of the renal ET system and tubular protein reabsorption. Exposure of proximal tubular cells *in vitro* to a protein load leads to a dose-dependent increase in ET-1 production (129). This phenomenon is not associated exclusively with albumin but may be seen with other proteins, such as IgG and transferrin (129). Animals that ingest dietary acid have increased accumulation of interstitial ET-1, suggesting that the consequent enhanced renal ET-1 production, possibly by renal tubular and/or renal endothelial cells, is abluminally secreted (130). Within the interstitium, ET-1 has the capacity to bind to interstitial fibroblasts and promote their proliferation and to generate extracellular matrix (131). Furthermore, ET-1 is chemotactic for blood monocytes (132), leading to secretion of proinflammatory cytokines and growth factors, events that could contribute to interstitial remodeling and scarring. Hence, a potential pathway may be envisaged whereby ET-1 could link proteinuria to interstitial fibrosis.

In the remnant kidney model, renal ET-1 gene expression and urinary ET-1 excretion correlate with degree of proteinuria and extent of renal damage (73). Also, transgenic animal studies in which renal ET pathways are upregulated display renal tubulointerstitial lesions independent of changes in BP (133). These BP-independent effects of ET are supported by antagonist studies in which ET receptor antagonists led to a slowing of progressive renal damage, even in the absence of BP modification (134). Nonselective $ET_{A/B}$ receptor antagonism can reduce the increase in collagen and extracellular matrix deposition that is seen in rats that are treated with NG-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, independent of BP changes, and can also reduce collagen I gene activity to normal levels, suggesting that ET-1 promotes renal fibrosis *via* activation of this gene (135). ET receptor antagonists have also been shown to attenuate the progression of renal insufficiency in a rat remnant kidney model (136). Although nonselective $ET_{A/B}$ receptor antagonists have produced positive results (137), the effect is greater with selective ET_A receptor antagonism (138). Indeed, concomitant administration of an ET_B receptor antagonist can abolish the beneficial effects of ET_A receptor antagonism (139). In patients with nephrotic syndrome as a result of focal and segmental glomerulosclerosis, plasma and urinary ET-1 concentrations are significantly higher than those in healthy control subjects (140), and nephrotic patients who show a reduction in proteinuria with immunosuppressive therapy also show reductions in urinary ET-1 excretion (141).

Blockade of the ET System and RAS: A Potential Synergism

ET-1 and AngII are powerful vasoconstrictors involved in the regulation of vascular tone, and there is considerable evidence for an interaction between the ET and RAS (142). AngII increases ET-1 transcription and secretion *in vitro* in a variety of cell types, including endothelial and vascular smooth muscle cells (143,144). ACE inhibitors also reduce renal ET-1 formation. Rats with reduced renal mass show a parallel fall in proteinuria, vascular and glomerular prepro-ET-1 mRNA, and ET-1 peptide after RAS blockade with losartan and captopril (145). Chronic ACE inhibitor treatment in animal models of glomerulosclerosis (146) and immune-mediated glomerulonephritis (147) leads to a reduction in proteinuria as well as a fall in renal ET-1 mRNA and protein expression.

It is interesting that animal data have suggested that concomitant acute blockade of the RAS and ET system produces greater hemodynamic changes than those seen with blockade of either system alone (148–152). Also, many clinical studies using ET receptor antagonists in patients with heart failure demonstrated major additional hemodynamic effects (153,154) in patients who already were receiving ACE inhibitors. Synergism with respect to acute systemic hemodynamic effects between ET_A receptor antagonists and angiotensin receptor type 1 antagonists (ARB) (51) or ACE inhibitors (53) has been demonstrated in humans. More recently, the combination of ET receptor antagonism and ACE inhibition has been shown to improve endothelial function (155,156).

With respect to the kidneys, when ET_A receptor antagonism is given in the presence of ACE inhibition in healthy subjects, contrary to a lack of effect of ET_A receptor antagonism alone, an increase in RBF and natriuresis is observed, an effect that seems

to be both ET_B dependent and NO mediated (53). Although it is tempting to attribute the increase in sodium excretion to the activity of an unblocked tubular ET_B receptor, it is possible that the natriuresis is entirely a consequence of the renal vasodilation and so essentially a hemodynamic effect. This would likely be the case if the intrarenal changes in RBF—of an ET_B-mediated increase in medullary blood flow—that are seen in animal models also occur in humans. Further human studies are needed to characterize the role of the renal ET_B receptor and the interaction between ET and AngII in CKD, in which there is increased activity in the RAS, and in a setting where many patients are already treated with ACE inhibitors.

ET Antagonism as a Treatment Strategy in CKD

ET-1 plays a role in the maintenance of BP and arterial stiffness. It also contributes to ED, oxidative stress, and vascular inflammation, with additional longer term effects on vascular remodeling. In animals, ET receptor antagonists have been shown to reduce BP, improve arterial stiffness and ED, and retard the progression of atherosclerosis. Some of these observations are confirmed by clinical studies. However, studies in CKD are fairly limited but suggest that, in addition to having a beneficial effect on systemic hemodynamics, ET receptor antagonists improve renal function and may potentially reduce renal disease progression.

The question of whether selective or nonselective receptor blockade should be used is probably disease specific and in CKD remains to be clarified. However, preliminary evidence in patients with CKD suggests that both selective ET_A and nonselective $ET_{A/B}$ receptor blockade reduces BP but that selective ET_A blockade has additional desirable effects on renal hemodynamics (52). From the current evidence base, concomitant ET_B receptor blockade seems at best to offer no advantage over selective ET_A antagonism and may potentially reduce the benefits. Further studies are needed to discern the theoretical beneficial effects of an unblocked ET_B receptor in terms of natriuresis, diuresis, and glomerular hemodynamics.

Currently, a phase III trial is in progress to explore the potential of a selective ET_A receptor antagonist, avosentan, in diabetic nephropathy (157), but more clinical data are essential to advancing our broader understanding of the role of ET receptor antagonism not only as a potential renoprotective therapy in CKD but also as a treatment for the CVD with which it is associated.

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