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REVIEW

Endothelin-1 and the kidney – beyond BP

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Since its discovery over 20 years ago endothelin-1 (ET-1) has been implicated in a number of physiological and pathophysiological processes. Its role in the development and progression of chronic kidney disease (CKD) is well established and is an area of ongoing intense research. There are now available a number of ET receptor antagonists many of which have been used in trials with CKD patients and shown to reduce BP and proteinuria. However, ET-1 has a number of BP-independent effects. Importantly, and in relation to the kidney, ET-1 has clear roles to play in cell proliferation, podocyte dysfunction, inflammation and fibrosis, and arguably, these actions of ET-1 may be more significant in the progression of CKD than its prohypertensive actions. This review will focus on the potential role of ET-1 in renal disease with an emphasis on its BP-independent actions.

Abbreviations

Ang II, angiotensin II; BP, blood pressure; CD, collecting duct; CKD, chronic kidney disease; DC, dendritic cell; DN, diabetic nephropathy; ET-1, enothelin-1; ET_A, GEnC, glomerular endothelial cell; GFR, glomerular filtration rate; Mφ, macrophage; MCP-1, macrophage chemotactic protein-1; MIP-1β, macrophage inflammatory protein-1β; RBF, renal blood flow; TLR, toll-like receptor

Introduction

Since its discovery in 1988 (Yanagisawa *et al.*, 1988b), endothelin (ET) has been widely implicated in the pathophysiology of renal disease. ETs are a family of three 21 amino acid peptides, each with distinct genes and tissue distributions, with powerful vasoconstrictor and pressor properties (Yanagisawa *et al.*, 1988b; Inoue *et al.*, 1989; Arinami *et al.*, 1991). Of the three peptides, ET-1 is the major endothelial isoform and, in the human kidney, the only one so far shown to be expressed at the protein level (Karet and Davenport, 1996). 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 (Eid *et al.*, 1994). Within the renal system, it is produced by glomerular epithelial and mesangial cells, renal tubular and medullary collecting duct (CD) cells (Kohan, 1997) and, potentially, resident and infiltrating macrophages (Ehrenreich *et al.*, 1990). Importantly, ET-1 has clear roles to play in cell proliferation, podocyte dysfunction, inflammation and fibrosis (Dhaun *et al.*, 2006), and arguably, these effects of ET-1 may be more significant in the progression of chronic kidney disease (CKD) than its prohypertensive actions. This review will focus on the potential role of ET-1 in renal disease with an emphasis on those actions independent of BP. As these data are largely derived from animal models, the focus will be on preclinical studies.

Biology of the ET system in the kidney

Regulation of ET synthesis occurs at the level of gene transcription, with the gene product being the 212 amino acid preproET-1. ET-1 has limited intracellular stores so is mostly

transcriptionally regulated. Enhanced gene transcription occurs with a wide range of stimuli (Wesson *et al.*, 1998; Attina *et al.*, 2005). Those pertinent to CKD include other vasocontrictors (Ang II, vasopressin), pro-inflammatory cytokines (TNF-α, IL-1b), hypoxia, reactive oxygen species, profibrotic cytokines (TGF-β, platelet-derived growth factor), hyperglycaemia, acidosis and thrombin. Thus, an up-regulation in ET-1 synthesis may be viewed as a common renal stress response. By contrast, prostacyclin, NO and the natriuretic peptides all inhibit gene transcription. PreproET-1 is cleaved to big ET-1 (38 amino acids), which is largely biologically inactive (Haynes and Webb, 1994). Endothelin converting enzyme then splits big ET-1 to the biologically active ET-1 and C-terminal fragment. Once synthesized, the secretion of mature ET-1 from endothelial cells is largely abluminal (Yoshimoto *et al.*, 1991), towards the adjacent vascular smooth muscle, suggesting an autocrine or paracrine mechanism of action.

The effects of ET-1 are mediated via two G-protein coupled receptors, the ET_A and ET_B receptor (Arai *et al.*, 1990; Sakurai *et al.*, 1990). Within blood vessels, both receptors are found on smooth muscle cells and their activation results in vasoconstriction. ET_B receptors are, however, predominantly found on the vascular endothelium where their activation results in vasodilatation via prostacyclin and NO (DeNucci *et al.*, 1988). Because most ET-1 is released abluminally, plasma concentrations of ET-1 do not accurately reflect ET-1 production. However, some is released into the circulation and the ET_B receptor also acts as a clearance receptor for this circulating ET-1. The half-life of ET-1 in the healthy circulation is ~1 min (Gasic *et al.*, 1992) 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 (Dupuis *et al.*, 1996), although the splanchnic and renal circulations also contribute (Attina *et al.*, 2005). Therefore, reductions in ET_B numbers, or ET_B receptor blockade, may reduce ET-1 clearance, increasing plasma concentrations without altering production. 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 (Karet and Davenport, 1996; Kuc and Davenport, 2004).

Effects of ET-1 on renal haemodynamics

ET-1 is a potent vasoconstrictor *in vitro* and pressor in whole animals (Yanagisawa *et al.*, 1988a). With respect to the kidney, exogenous ET-1 causes renal vasoconstriction and an overall reduction in RBF (Chou and Porush, 1995), effects mediated via the ET_A receptor (Evans *et al.*, 2001; Abassi *et al.*, 2002). Indeed, the renal vasculature is more sensitive to the vasoconstricting effects of ET-1 than other vascular beds (Pernow *et al.*, 1989). Although exogenous ET-1 reduces total RBF, a regional difference has been observed, with cortical vasoconstriction that is ET_A receptor mediated (Rubinstein *et al.*, 1995; Gurbanov *et al.*, 1996; Denton *et al.*, 2004), and medullary vasodilatation which is ET_B and NO dependent (Rubinstein *et al.*, 1995). Furthermore, *in vitro* studies have shown that combined $ET_{A/B}$ receptor antagonism is required to abolish the vasoconstricting effects of exogenous ET-1 on the afferent arteriole suggesting that both ET_A and ET_B receptors are involved (Inscho *et al.*, 2005). At the efferent arteriole, the actions 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 (Inscho *et al.*, 2005). By this action on efferent and afferent arterioles, ET has the ability to regulate glomerular capillary pressure and GFR.

In healthy man, a similar peripheral vasoconstrictor (Haynes and Webb, 1994) and systemic pressor response has been demonstrated (Sorensen *et al.*, 1994), as well as renal vasoconstriction, a fall in total RBF (with a consequent reduction in GFR) and increase in filtration fraction (Rabelink *et al.*, 1994). As yet, there are no studies of the effects of ET-1 on intra-renal distribution of blood flow in man. Also, there are few studies using ET receptor antagonists. One has demonstrated an increase in RBF after combined $ET_{A/B}$ receptor blockade (Freed *et al.*, 1999). Most, however, do not demonstrate an effect of selective ET_A receptor blockade (Schmetterer *et al.*, 1998; Montanari *et al.*, 2000; 2002; Goddard *et al.*, 2004a,b), or combined $ET_{A/B}$ receptor blockade (Goddard *et al.*, 2004b), on basal renal haemodynamics, 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 can, however, produce profound renal vasoconstriction, suggesting that ET-1 mediated tonic renal vasodilatation via the ET_B receptor is important (Goddard *et al.*, 2004b).

Studies in patients with CKD are limited. In the renal circulation, in contrast to healthy controls, acute selective ET_A but not combined $ET_{A/B}$ receptor antagonism produces a sustained increase in RBF and a decrease in renal vascular resistance suggesting that ET-1 is important in maintaining renal vascular tone (which is about four times higher at baseline than in healthy controls) via the ET_A receptor. Because GFR does not significantly alter, this is accompanied by a reduction in filtration fraction that, in the absence of changes in filtration coefficient, may indicate efferent arteriolar dilatation, consistent with animal data suggesting a role for ET-1 in maintaining efferent arteriolar tone via the ET_A receptor (Goddard *et al.*, 2004b). Interestingly, chronic ET_A receptor antagonism is associated with no significant changes in RBF but a fall in GFR (Dhaun *et al.*, 2011), effects similar to those seen with chronic angiotensin converting enzyme (ACE) inhibition.

The attenuation of the acute renal vasodilatory effect of ET_A receptor antagonism by concomitant ET_B receptor antagonism suggests that the ET_B receptor is important in maintaining renal vasodilatation in CKD and that the renal vasoconstriction seen after selective ET_B receptor blockade alone is not simply due to reduced clearance and displacement of ET-1 onto the unblocked ET_A receptor but due to a specific role for ET-1 mediated tonic renal vasodilatation via

the ET_B receptor (Goddard *et al.*, 2004b). Studies in healthy subjects have suggested that this observation in CKD patients may be accounted for at least in part by concomitant administration of ACE inhibitors as these are synergistic with ET_A receptor antagonism via an NO mediated, ET_B receptor-dependent mechanism (Schmetterer *et al.*, 1998; Montanari *et al.*, 2000; 2002; Goddard *et al.*, 2004a,b).

Effects of ET-1 on the glomerulus (see Figure 1)

The glomerulus is the functional filtration unit of the kidney. The glomerular filtration barrier – composed of two cells types: fenestrated endothelial cells (making up the glomerular capillaries) and podocytes – allow the filtration of water and small molecules whilst excluding large proteins. This barrier is size and charge selective. Sandwiched between the endothelial cells and podocytes is the glomerular basement membrane to which both cell types contribute. It is likely that all components of the glomerular filtration barrier have a role in enabling ultrafiltration and there is considerable crosstalk between the podocyte and endothelium (Mathieson, 2012). Supporting the glomerular capillaries are specialized pericytes known as mesangial cells. These produce extracellular matrix and act as 'support' structures within the glomerulus. They also have a contractile function important in changing the filtration coefficient (Guo and Cantley, 2010).

Glomerular injury is frequently associated with progressive CKD. It ranges from the indolent progressive injury of diabetes and hypertension to acute severe inflammation found in systemic vasculitis or lupus nephritis. CKD typically involves glomerular sclerosis and interstitial fibrosis and these may occur regardless of the nature of the initial renal insult. The mechanisms responsible for this continued renal deterioration are not fully understood, but likely involve a number of common pathways and may be distinct from those responsible for the original injury. Glomerular hypertension, glomerular cell hypertrophy and extracellular matrix accumulation are all involved. Significant proteinuria, a marker of CKD, has emerged as a powerful predictor of renal disease progression (Cameron *et al.*, 1978; Mallick *et al.*, 1987) regardless of underlying diagnosis, and proteinuria reduction is an important strategy to retard or prevent loss of renal function (Ruggenenti *et al.*, 2003). Furthermore, reduction of proteinuria confers cardiovascular protection (Ibsen *et al.*, 2005). Once filtered through the glomerulus excess protein is tubulo-toxic and excess protein reabsorption in the tubules can lead to an activation of tubular-dependent pathways of interstitial inflammation and fibrosis, with progressive renal scarring (Remuzzi and Bertani, 1998).

The ET system has been implicated in these processes (Kohan, 1997). In the remnant kidney model, renal ET-1 gene expression and urinary ET-1 excretion correlate with the degree of proteinuria and extent of renal damage (Orisio *et al.*, 1993). Also, transgenic animals in which renal ET pathways have been up-regulated, display glomerulosclerosis and renal tubulointerstitial lesions independent of changes in BP that are usually characteristic of such models (Hocher *et al.*, 1997). These BP-independent effects of ET-1 are supported by antagonist studies where ET receptor antagonists lead to a slowing of progressive renal damage, even in the absence of BP modification (Benigni and Remuzzi, 1999). Furthermore, infusion of ET-1 into rats over 2 weeks increases the perme-

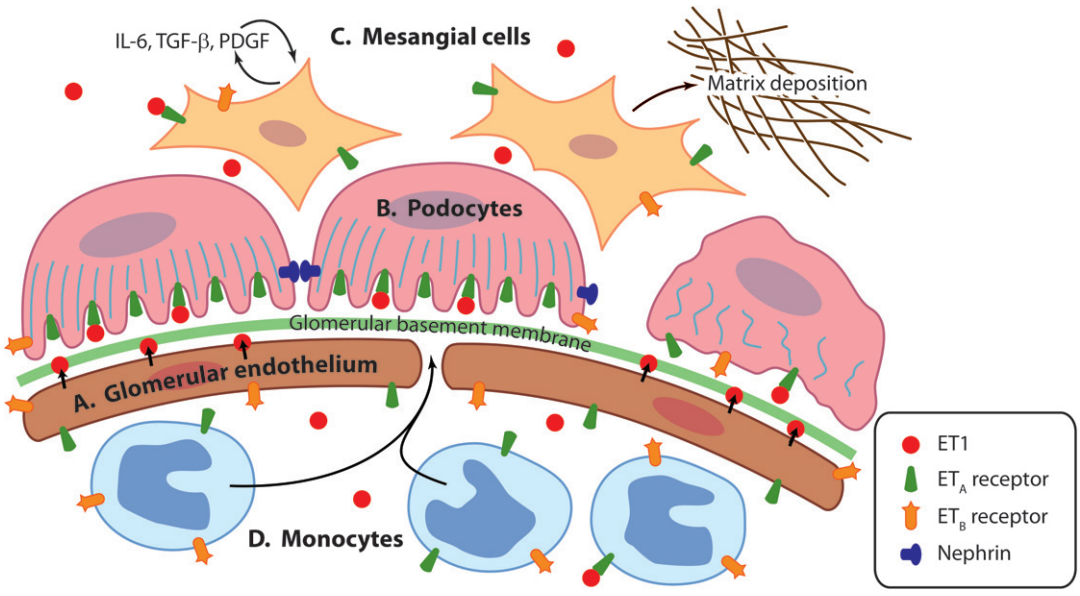

Figure 1

ET-1 effects on the glomerulus. (A) ET-1 is secreted on abluminal surface of glomerular endothelial cells. (B) ET-1 causes contraction of podocyte actin cytoskeleton and loss of slit diaphragm proteins such as nephrin. (C) Mesangial cells are activated to produce pro-inflammatory cytokines and matrix proteins. (D) ET-1 acts as a chemoattractant to monocytes.

ability of isolated glomeruli to albumin and this effect was blocked by ET_A receptor antagonism (Saleh *et al.*, 2010).

Podocytes

Podocytes are complex epithelial cells consisting of a cell body from which major 'primary' processes arise. These in turn give rise to smaller 'secondary' foot processes. Secondary processes from adjacent podocytes interdigitate and form a 'zipper-like' connection known as the slit diaphragm. This slit diaphragm is maintained by multiple protein-protein interactions, which connect and signal with the podocyte actin cytoskeleton (Greka and Mundel, 2012). Podocyte injury is seen in many human glomerular diseases including diabetic nephropathy (DN), minimal change disease, focal segmental glomerulosclerosis and membranous nephropathy, and leads to proteinuria. The earliest pathological sign of podocyte injury is foot process effacement as connections between podocytes are lost. This may be followed by cell detachment and loss with eventual glomerulosclerosis. The presence of podocytes in the urine may be a useful indicator of disease severity (Greka and Mundel, 2012) and there is increasing interest in therapy that specifically targets and protects the podocyte (Mathieson, 2012).

Podocytes have been shown to both express ET-1 and to have ET receptors. Separate data support the presence of ET_A (Morigi *et al.*, 2005) and ET_B (Yamamoto *et al.*, 2002) receptors on rodent podocytes, although it is likely that they possess both (Davenport *et al.*, 1989). For human podocytes, there are data from agonist-antagonist studies suggesting that ET_A receptors are present (Spath *et al.*, 1995; Collino *et al.*, 2008). However, it is likely that human podocytes also possess both ET_A and ET_B receptors (Rebibou *et al.*, 1992).

Treatment of mouse podocytes with albumin or IgG leads to disruption of the actin cytoskeleton, activation of focal adhesion kinase, which activates transcription of preproET-1 mRNA leading to ET-1 secretion (Morigi *et al.*, 2005). Similarly, treatment of podocytes with shiga toxin leads to ET-1 secretion. Shiga toxin is the offending agent of post-diarrhoeal haemolytic-uraemic syndrome, which is characterized by glomerular ischaemic changes preceding microvascular thrombosis. In turn, ET-1, acting in an autocrine manner via the ET_A receptor, causes disruption of the podocyte actin cytoskeleton (Morigi *et al.*, 2006).

One of the key podocyte proteins at the slit diaphragm is nephrin and mutations of the nephrin gene lead to congenital nephrotic syndrome (Kestila *et al.*, 1998). In a number of human glomerular diseases, nephrin is either down-regulated or its cellular localization altered (Welsh and Saleem, 2010). Nephrin is often used in experimental models as a marker of podocyte injury. There are limited *in vitro* data suggesting that ET-1 may lead to podocyte loss of nephrin. Treatment of podocytes with exogenous ET-1 reduced cell surface nephrin expression. This podocyte shedding of nephrin was blocked by an ET_A receptor antagonist (Collino *et al.*, 2008). Similarly, infusion of ET-1 into rats caused nephrin excretion into the urine, again inhibited by ET_A receptor antagonism (Saleh *et al.*, 2010).

The role of ET-1 in podocyte injury in CKD has been recently reviewed (Fligny *et al.*, 2011) so will only be briefly discussed here. In a number of experimental models of renal injury, there is evidence that the beneficial effects of ET antagonists may be, in part, due to protection of podocyte structure and function. Aged Wistar rats spontaneously develop glomerulosclerosis and proteinuria associated with electron micrograph evidence of podocyte injury. These changes can be reduced by treatment with an ET_A receptor antagonist in the absence of a fall in BP (Ortmann *et al.*, 2004). More recently, in separate studies in streptozocin-induced DN, treatment with an ET_A receptor antagonist reduced BP and proteinuria. These effects were associated with either preservation of podocyte number (Gagliardini *et al.*, 2009) or maintenance of glomerular nephrin expression and a reduction of nephrin loss in the urine (Saleh *et al.*, 2011). Future studies are likely to focus on changes in podocyte number and phenotype, as more effective antibodies are available for immunohistochemistry. This will include markers such as nephrin, synaptopodin and podocin, all of which contribute to the machinery of the slit diaphragm (Mathieson, 2012).

Taken together, the current data suggest that podocytes release, bind and respond to ET-1. ET-1 can induce cytoskeletal remodelling in podocytes but how such changes would affect GFR and RBF remains speculative. Furthermore, whilst ET-1 may increase podocyte protein permeability, whether water and small solute filtration will be increased remains speculative. Finally, although putative effects of ET-1 and ET receptor antagonism have been demonstrated *in vitro*, no direct effects of ET-1 on podocytes have been demonstrated *in vivo*.

Glomerular endothelium

The glomerular endothelium is a specialized fenestrated capillary bed. Its role in the development of renal disease and proteinuria has been less well studied than that of the podocyte due to difficulty in culturing these cells *in vitro* and the lack of glomerular endothelial-specific gene targeting approaches. Glomerular endothelial cells (GEnC) are probably the principal source of ET-1 within the glomerulus (Herman *et al.*, 1998), and glomerular ET-1 staining is increased in the presence of proteinuric renal disease including IgA nephropathy, lupus nephritis and membranous nephropathy (Lehrke *et al.*, 2001). It is probable that ET-1 secreted abluminally from GEnC modulates podocyte and mesangial cell structure and function. However, study in this area is limited. Recent data have shown that conditioned media from GEnC stimulated with serum from patients with pre-eclampsia cause podocyte nephrin shedding and changes in the actin cytoskeleton *in vitro* (Collino *et al.*, 2008). An ET_A receptor antagonist was able to inhibit these changes, although the authors did not show evidence for the direct production of ET-1 by GEnC.

Mesangial cells

Mesangial cells are glomerular pericytes and they act to support the other glomerular structures. They synthesize mesangial matrix, which is the 'filler' between adjacent mesangial cells. Mesangial cells are able to produce a significant amount of ET-1 (Sakamoto *et al.*, 1990; Herman *et al.*, 1998), which is increased by vasoactive substances such as Ang II and vasopressin (Ikeda *et al.*, 1995), TGF-β (Zoja *et al.*, 2011) and TNF-α (Kohan, 1997). These cells express both ET_A

and ET_B receptors and are ET-1 responsive (Takeda *et al.*, 1994; Herman *et al.*, 1998; Orth *et al.*, 2000). ET-1 can itself stimulate mesangial cell ET-1 production (Iwasaki *et al.*, 1995), and in rats, this has been shown to be mediated via the ET_B receptor (Iwasaki *et al.*, 1995). ET-1 stimulates contraction of mesangial cells *in vitro* (Simonson *et al.*, 1989) as well as in isolated glomeruli (Saleh *et al.*, 2010). These effects of ET-1 would result in changes in glomerular filtration area and intraglomerular haemodynamics.

Many glomerular diseases are associated with increased mesangial matrix and fibrosis (glomerulosclerosis), with DN being the most common. Chronic ET-1 overexpression leads to glomerulosclerosis without systemic hypertension (Hocher *et al.*, 1997). Stimulation of mesangial cells with ET-1 increases expression of collagen types I and IV, fibronectin and versican all of which contribute to extracellular matrix. ET-1 also increases mesangial cell production of tissue inhibitor of matrix metalloproteinase 3 and plasminogen activator inhibitor 2, which inhibit mesangial matrix degradation (Gomez-Garre *et al.*, 1996; Mishra *et al.*, 2003). Furthermore, ET-1 stimulates mesangial cell production of the cytokines TGF-β, IL-6, osteopontin and MCP-1, which results in an autocrine signalling loop increasing collagen synthesis. These effects of ET-1 on mesangial cells appear to be mediated via the ET_A receptor (Simonson and Ismail-Beigi, 2011).

Mesangial cell proliferation is common in many glomerulonephritides, such as IgA nephropathy and lupus nephritis. ET-1 stimulates mesangial cell mitogenesis (Simonson *et al.*, 1989; Gomez-Garre *et al.*, 1996). This response is largely mediated via the ET_A receptor. However, there are data from cultured human mesangial cells suggesting that the ET_B receptor may also be involved (Orth *et al.*, 2000). ET-1 also stimulates mesangial cell production of platelet-derived growth factor, leading to an autocrine loop of cell proliferation and further secretion (Jaffer *et al.*, 1990; Ikeda *et al.*, 1995). Thus, ET-1 can alter the structure and function of glomerular mesangial cells, independent of changes in BP. These effects of the ET system are likely to contribute to renal disease progression. However, it must be noted that any conclusions about the role of ET-1 in mesangial cell biology are based entirely on *in vitro* data. There remains no conclusive demonstration of a physiological role for ET-1 mediated mesangial cell contraction and so it is likely that any functional or pathological importance of mesangial cell-derived ET-1 will remain speculative.

Effects of ET-1 on the tubule

With respect to renal tubular functions, there is now a substantial body of evidence supporting a role for ET-1 in the regulation of salt and water homeostasis. This has been reviewed extensively recently (Kohan *et al.*, 2011). *In vitro*, sarafotoxin 6c, a selective ET_B receptor agonist, inhibited chloride transport in cells from the thick ascending loop of Henle. This was in an equipotent manner to ET-1 and was inhibited by an ET_B receptor antagonist, but not an ET_A antagonist (Plato *et al.*, 2000). Animal data also support a role for the ET_B receptor in natriuresis and diuresis. 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 CD cells (Gariepy *et al.*, 2000). Antagonist studies have also proved helpful with ET_B antagonist-treated rats developing a sodium-dependent hypertension (Webb *et al.*, 1998; Pollock, 2001).

However, recent *in vivo* studies have explored the possible roles of the ET_A receptor in the effects of ET-1 in the renal medulla. CD ET-1 knockout (KO) (Ahn *et al.*, 2004) and CD ET_B KO (Ge *et al.*, 2006) mice show higher BP and salt sensitivity compared with wild type mice. However, the magnitude of these effects in CD ET-1 KO mice is almost double that in CD ET_B KO mice, indicating that CD-derived ET-1 may have multiple targets, most likely either ET_B receptors on other cell types or ET_A receptors. Interestingly, CD ET_A KO mice are not hypertensive, even under a high-salt diet (Ge *et al.*, 2005). Surprisingly, the BP of mice with both ET_A and ET_B receptors knocked out in CD (CD $ET_{A/B}$ KO mice) is almost identical to that of CD ET-1 KO mice (Ge *et al.*, 2008). Although the specific mechanisms for this phenomenon are still under investigation, the authors of that study speculated that ET_A receptors in the CD help maintain salt balance if the ET_B receptor is dysfunctional. The natriuretic ability of ET_B receptors may compensate for (or overwhelm) the lack of ET_A activity in CD ET_A KO mice. In support of this hypothesis, ET-1 infused into the renal medulla of female ET_B receptor-deficient rats caused ET_A receptor-dependent natriuresis (Nakano and Pollock, 2009). However, dissecting the different actions of the intrarenal ET system has proved difficult, in part due to an inability to discriminate between effects of ET-1 *in vivo* on the nephron and on the vasculature.

Effects of ET-1 on renal inflammation

Inflammation contributes to the development and progression of CKD as well as the incident cardiovascular disease with which CKD is associated. In some conditions there is a transient and acute inflammatory response that leads to impaired renal function, for example, anti-neutrophil cytoplasmic antibody-associated vasculitis, lupus nephritis and interstitial nephritis. However, even in more smouldering renal diseases, such as membranous, IgA or DN, there is a correlation between the presence of low level inflammatory infiltrates and progression to end-stage renal disease (Bohle *et al.*, 1992; Radford *et al.*, 1997; Kelly and Dominguez, 2010).

ET-1 likely contributes to the development of inflammation. Systemic overexpression of ET-1 leads to renal, cardiac and pulmonary inflammation and fibrosis (Hocher *et al.*, 1997; 2000). Furthermore, endothelial-restricted ET-1 overexpressing mice develop evidence of vascular inflammation in the absence of hypertension (Amiri *et al.*, 2008). ET-1 also mediates renal inflammation induced by Ang II. In mice, chronic infusion of Ang II causes hypertension and a T-cell-rich renal infiltrate. Treatment with an ET_A receptor antagonist reduced BP and attenuated the numbers of T cells in the renal cortex. Interestingly, alternative methods of reducing BP, which achieved similar BP control, did not significantly affect T-cell infiltration (Boesen *et al.*, 2011). Similarly, aldosterone-treated rats developed hypertension and renal macrophage (Mφ) infiltration that was reduced by ET_A

receptor antagonism (Tostes *et al.*, 2002). The mechanism for these changes is not clear but should be the focus for future research.

There is evidence that leucocytes are able to synthesize and regulate ET-1 production. Polymorphonuclear neutrophils produce proteolytic enzymes that convert big ET-1 to the active peptide and are subsequently able to break this down (Patrignani *et al.*, 1991; Kaw *et al.*, 1992). Mast cells increase in number in many renal inflammatory kidney diseases and may have a role in limiting renal injury and restoring homeostasis (Blank *et al.*, 2007). Regulating the effects of ET-1 may form part of this mechanism. In support of this hypothesis, mast cells have been shown to limit ET-1 action *in vivo* both by receptor-mediated uptake and through the action of proteolytic enzymes (Maurer *et al.*, 2004).

There is convincing evidence that Mφs are able to secrete ET-1. Original studies showed that human monocyte-derived Mφs produce ET-1 following LPS stimulation (Ehrenreich *et al.*, 1990), although other studies have not confirmed this (Spirig *et al.*, 2009). Human alveolar Mφs produce ET-1 on stimulation with thrombin or LPS (Kobayashi *et al.*, 1997), and increased ET-1 secretion was found in alveolar Mφ isolated from patients with scleroderma and pulmonary fibrosis (Odoux *et al.*, 1997). Dendritic cells (DCs) are the major type of antigen presenting cell and can develop from Mφ. Human DCs produce ET-1 and synthesis is increased by stimulation with LPS and lipotechoic acid [exogenous toll-like receptor (TLR) 4 and TLR2 ligands], and hyaluronic acid and heparan sulphate (endogenous TLR ligands) (Guruli *et al.*, 2004; Spirig *et al.*, 2009). Overall, Mφs are able to produce ET-1 when activated but there is no convincing evidence that they provide a significant source of ET-1 in renal injury. This is based on the observation that in renal biopsy studies Mφ or other leucocytes do not significantly express ET-1 or its receptors (Lehrke *et al.*, 2001).

ET-1 exerts a number of pro-inflammatory effects. It acts as a chemo-attractant for PMN (Wright *et al.*, 1994; Cui *et al.*, 2001) and Mφ (Achmad and Rao, 1992). When assessed, chemokinesis appears to be inhibited by blockade of the ET_A receptor. ET-1 can also activate the endothelium to increase leucocyte adhesion and transmigration both *in vitro* (Zouki *et al.*, 1999) and *in vivo* (Callera *et al.*, 2004). ET-1 also stimulates secretion of MCP-1 from mesangial cells, which is chemotactic to Mφ (Ishizawa *et al.*, 2004). Finally, ET-1 may also promote DC differentiation (Guruli *et al.*, 2004). Although both ET_A and ET_B receptors have been identified on Mφ (Bacon *et al.*, 1996; Mencarelli *et al.*, 2009), their respective roles remain unclear. There is some evidence that ET-1 can elicit classical pro-inflammatory Mφ activation with activation of the NF-κB signalling pathway and release of TNF-α (Wilson *et al.*, 2001; Juergens *et al.*, 2008), although this result has not been consistently observed (Speciale *et al.*, 1998; Spirig *et al.*, 2009). ET-1 does stimulate Mφ production of the chemokine macrophage inflammatory protein-1β. These changes may be part of an ET-1-induced alteration in Mφ phenotype. However, data to this end are lacking but would be of great interest given the potential role for Mφ in renal repair (Kluth, 2007). Overall, the main effect of ET-1 on leucocytes is to promote a chemotactic response that serves to increase the leucocyte infiltrate within the kidney.

ET antagonism in models of renal disease

A number of ET receptor antagonists have been used in animal models of renal disease. These show a range of potentially beneficial effects many of which are unlikely to be a result of the BP lowering effects of these drugs. An important point when considering these data is to remember that ET antagonists may selectively block the ET_A receptor or act to provide mixed $ET_{A/B}$ receptor antagonism. Benefits of one approach over the other have been previously discussed (Dhaun *et al.*, 2007; Schneider *et al.*, 2007).

Hypertensive nephropathy

Hypertensive nephropathy (or nephrosclerosis) is characterized histologically by vascular, glomerular and tubulointerstitial involvement. The vascular disease consists of intimal thickening and luminal narrowing of the large and small renal arteries and the glomerular arterioles. The glomeruli may show both focal global (involving the entire glomerulus) and focal segmental sclerosis. The vascular and glomerular diseases may be associated with an often severe interstitial nephritis.

The Benigni group was amongst the first to report the benefits of ET receptor blockade in hypertensive nephropathy. They reported reductions in proteinuria and glomerulosclerosis after selective ET_A receptor blockade in a renal mass reduction rat model of hypertensive nephropathy (Benigni *et al.*, 1993). Following a different approach, Hocher *et al.*, (1997) showed that systemic overexpression of the preproET gene in mice led to glomerulosclerosis and interstitial fibrosis in the absence of a change in BP (Hocher *et al.*, 1997). Since these early studies, many other preclinical data have emerged confirming the renoprotective effects of both ET_A and $ET_{A/B}$ receptor antagonism in several forms of hypertension – Ang II-dependent, renin-dependent, salt-loaded renin-dependent, aldosterone-induced, genetically salt-sensitive, deoxycorticosterone acetate-salt induced – and these are reviewed in Schiffrin (2005).

The work by Opocensky *et al.* (2006) has shown that in hypertensive rats podocyte injury preceded proteinuria. The selective ET_A antagonist atrasentan, but not the mixed $ET_{A/B}$ antagonist bosentan, prevented podocyte injury and substantially reduced proteinuria despite the fact that animals were still markedly hypertensive (Opocensky *et al.*, 2006). Importantly, treatment was begun after hypertension had been established, and the beneficial effects on podocyte injury were seen at an early stage of renal injury, that is in the absence of established glomerulosclerosis. Podocyte injury prior to the development of glomerulosclerosis was also observed in Dahl hypertensive rats, and this was sensitive to mineralocorticoid receptor blockade (Nagase *et al.*, 2006). Importantly, unlike ET or mineralocorticoid receptor blockade, antihypertensive treatment with hydralazine had no effect on podocyte injury, again indicating that the renoprotective effects are specific and largely pressure independent (Nagase *et al.*, 2006). Interestingly, Boffa *et al.* (2001) have shown *reversal* of vascular fibrosis and collagen deposition in a model of NO-deficient hypertension following ET receptor blockade.

Table 1

Models of glomerulonephritis and the effects of ET receptor antagonism

Model	Selective ET_A or mixed $ET_{A/B}$ antagonism	Effects	Reference
Immune complex GN (rat)	Mixed $ET_{A/B}$	Reduction in proteinuria and glomerular injury	(Gomez-Garre *et al.*, 1996)
Anti-Thy1 nephritis (characterized by acute mesangiolysis followed by mesangial proliferation; rat)	Selective ET_A	Reduced glomerular cell proliferation No assessment of inflammation, injury or proteinuria	(Fukuda *et al.*, 1996)
Membranous nephropathy (rat)	ACE inhibitor and ET_A receptor antagonist: alone and in combination	Reduced proteinuria Lower serum creatinine Less glomerular and tubulointerstitial injury Combination therapy superior to either intervention alone	(Benigni *et al.*, 1998b)
Heyman nephritis (rat)	ACE inhibitor, ARB, ET_A receptor antagonist: alone and in combination	Similar BP reduction ACE inhibitor superior in reducing proteinuria and glomerulosclerosis Additional effects of adding ET_A antagonist to either ACE inhibitor or ARB	(Amann *et al.*, 2001)

ARB, angiotensin receptor blocker.

Diabetic nephropathy

DN is now the commonest cause of end-stage renal failure. A number of studies have assessed the effects of different ET antagonists in models of DN with the majority using streptozocin to induce diabetes mellitus. Early studies with mixed $ET_{A/B}$ antagonists showed a fall in proteinuria and, where measured, BP (Benigni *et al.*, 1998a; Hocher *et al.*, 2001) There was also evidence of reduced glomerulosclerosis (Hocher *et al.*, 2001). An important comparator in such studies is the use of ACE inhibitors, which are the mainstay of treatment for DN. When assessing proteinuria (or albuminuria) none of the studies show that ET receptor antagonists are superior to ACE inhibitors. Some studies show equivalent efficacy (Gagliardini *et al.*, 2009; Watson *et al.*, 2010), whereas others show that ACE inhibitors are better (Zoja *et al.*, 2011). The combination of ACE inhibition with ET receptor antagonism may have additional advantages. In uninephrectomized streptozocin-induced diabetic rats treatment with lisinopril and avosentan normalized the proteinuria, reduced interstitial and glomerular scarring, prevented the loss of podocytes and maintained expression of nephrin (Gagliardini *et al.*, 2009). Here combination therapy was more effective than either drug alone.

ET receptor antagonists have also been shown to have a number of effects on markers of glomerular injury and inflammation in models of DN. Treatment with the ET_A receptor antagonist atrasentan, in streptozocin-induced diabetes, reduced albumin permeability in isolated glomeruli. In addition, treatment reduced expression of intercellular adhesion molecule 1 and MCP-1, whilst preserving expression of nephrin (Saleh *et al.*, 2010). Furthermore, the selective ET_A receptor antagonist sitaxentan reduced renal Mφ infiltration and expression of pro-inflammatory cytokines in different models of DN (Sasser *et al.*, 2007; Zoja *et al.*, 2011). These BP-independent, anti-inflammatory and podocyte protective effects of ET receptor antagonism may provide additional longer-term benefits in DN.

Glomerulonephritis

Glomerular disease outside of diabetes and hypertension has been less extensively studied with respect to ET receptor blockade. In particular, there are very few studies in inflammatory glomerulonephritis. The major studies of note are outlined in Table 1. Whether ET receptor antagonists are able to alter Mφ infiltration and function, podocyte biology and mesangial cell responses in glomerulonephritis has not been assessed.

Clinical studies

Most of the clinical studies in CKD relating to ET receptor antagonism have focussed on changes in BP and proteinuria and have shown reductions in both. These have been reviewed elsewhere (Dhaun *et al.*, 2006; Barton, 2008) and suggest that both selective ET_A and mixed $ET_{A/B}$ antagonist approaches may be of benefit in clinical CKD. There is only one study that relates to renal inflammation. This suggests that urinary ET-1, which is a measure of renal ET-1 synthesis (Benigni *et al.*, 1991; Dhaun *et al.*, 2009), is increased in those with active renal inflammation (Dhaun *et al.*, 2009). Interest-

ingly, urinary ET-1 levels fell following successful treatment of the inflammation. These data, however, were limited to those with lupus nephritis. It would be interesting to see if the same holds true for those patients with other forms of renal inflammatory disease such as small vessel vasculitis or interstitial nephritis. Furthermore, whether urinary ET-1 may be a useful biomarker of disease relapse in these conditions remains unclear.

Most clinical studies of ET receptor antagonists have restricted themselves to diabetic renal disease using BP and proteinuria as their end points. Future studies in patients with non-diabetic renal disease are now needed. Furthermore, the impact of ET receptor antagonists on clinical end points other than BP and proteinuria would be of great interest – some potential candidates include changes in urinary biomarkers (such as neutrophil gelatinase-associated lipocalin and kidney injury molecule-1), histological renal injury and novel cardiovascular risk factors (such as arterial stiffness, endothelial dysfunction, serum urate and asymmetric dimethylarginine).

Conclusion

The role of ET-1 in regulating BP and renal haemodynamics is well established. The paracrine effects of ET-1 that are likely to be important in the development and progression of CKD are less clearly understood. Renal ET-1 production is increased in most causes of renal injury. Glomerular endothelial cells are probably the principal source of renal-derived ET-1 and this may affect a number of local cell types, including podocytes, mesangial cells, other glomerular endothelial cells and inflammatory cells. Currently available data suggest that the majority of the pathological effects of ET-1, at least within the kidney, are mediated via the ET_A receptor. On podoytes, ET-1 causes alterations in the actin cytoskeleton, foot process effacement and loss of proteins such as nephrin that maintain the slit diaphragm. This contributes to the development of proteinuria, a powerful marker of renal disease progression. ET-1 activates mesangial cells to release pro-inflammatory and profibrotic cytokines; it stimulates cell proliferation and increases production of matrix proteins that can lead to glomerular sclerosis. ET-1 is also chemoattractant to leucocytes including Mφs, which infiltrate the glomerulus or interstitium and may further contribute to renal inflammation. More studies – both preclinical and clinical – are needed to further define the effects of ET-1 on these different renal cell types and to establish whether ET receptor antagonism may be of benefit in inhibiting inflammation and maintaining glomerular architecture.

Conflicts of interest

DCK has no conflict of interest. ND and DJW have received research grants from Pfizer. DJW has acted as a consultant to Pfizer.

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