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REVIEW

ET-1 actions in the kidney: evidence for sex differences

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Hypertension and chronic kidney disease are more common in men than in premenopausal women at the same age. In animal models, females are relatively protected against genetic or pharmacological procedures that produce high blood pressure and renal injury. Overactivation or dysfunction of the endothelin (ET) system modulates the progression of hypertension or kidney diseases with the ET_A receptor primarily mediating vasoconstriction, injury and anti-natriuresis, and ET_B receptors having opposite effects. The purpose of this review is to examine the role of the ET system in the kidney with a focus on the inequality between the sexes associated with the susceptibility to and progression of hypertension and kidney diseases. In most animal models, males have higher renal ET-1 mRNA expression, greater ET_A-mediated responses, including renal medullary vasoconstriction, and increased renal injury. These differences are reduced following gonadectomy suggesting a role for sex hormones, mainly testosterone. In contrast, females are relatively protected from high blood pressure and kidney damage via increased ET_B versus ET_A receptor function. Furthermore, ET_A receptors may have a favourable effect on sodium excretion and reducing renal damage in females. In human studies, the genetic polymorphisms of the ET system are more associated with hypertension and renal injury in women. However, the knowledge of sex differences in the efficacy or adverse events of ET_A antagonists in the treatment of hypertension and kidney disease is poorly described. Increased understanding how the ET system acts differently in the kidneys between sexes, especially with regard to receptor subtype function, could lead to better treatments for hypertension and renal disease.

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Abbreviations
ADPKD, autosomal-dominant polycystic kidney disease; AKI, acute kidney injury; Ang, angiotensin; CD, collecting duct; DOCA, deoxycorticosterone acetate; ECE, endothelin-converting enzyme; ESRD, end-stage renal disease; ET, endothelin; IMCD, inner medullary collecting duct; I/R, ischaemia/reperfusion; NO, nitric oxide; S6c, sarafotoxin 6c; TAL, thick ascending limb

The endothelin (ET) system plays a physiological role in regulating kidney function and blood pressure control. Overactivation or dysfunction of the ET system contributes to the development and progression of hypertension and kidney disease. Sex differences in cardiovascular and kidney diseases have been widely investigated and sex steroids influence almost every component of the ET system (Tostes *et al.*, 2008). Furthermore, ET-1 mediates the sex differences in the progression and development of hypertension and renal dysfunction. Because sex differences in general cardiovascular function of the ET system have been reviewed elsewhere (Tostes *et al.*, 2008), the present review will focus on the role of the ET system in control of renal function and what is currently known about sex differences in kidney-related disease, including hypertension and renal damage, in response to ET system activation or dysfunction. It is important to note that the majority of studies involving regulation of renal function by the ET system have been conducted in male animals, although some experiments do not identify the sex of the animals. For the purposes of the current review, we will assume that the sex of the animal or cells has not been indicated unless otherwise noted.

Components of the ET system

ET-1 has been described as the most potent vasoconstrictor substance. Hickey *et al.* first reported the existence of a peptidic substance released from cultured porcine aortic endothelial cells acting as an endothelial-derived contracting

factor (Hickey *et al.*, 1985). Yanagisawa *et al.* isolated and sequenced this novel peptide and gave it the name, endothelin (Yanagisawa *et al.*, 1988b). ET-1 is a 21-amino acid peptide with a hydrophobic C-terminal region and two inter-chain sulphide bonds between cysteine residues residing near the N-terminus, containing a highly conserved structure among species, including human, porcine and rat (Yanagisawa *et al.*, 1988a). Two structurally related peptides named ET-2 and ET-3 are encoded by separate genes and contain two- and six-amino acids different from ET-1 (Inoue *et al.*, 1989a). Moreover, the three ET isopeptides have structures and biological activity similar to the sarafotoxins, a family of isopeptides isolated from the venom of the snake *Atractaspis engaddensis* (Sokolovsky, 1992).

A bolus injection of any of the three ET isopeptides produces an initial transient depressor response followed by a prolonged increase in blood pressure, which can last as long as 30 min (Yanagisawa *et al.*, 1988b; Inoue *et al.*, 1989a). All three peptides are roughly equipotent in inducing the early transient depressor response. However, the pressor effect is much longer in ET-1 and ET-2 compared with ET-3 (Inoue *et al.*, 1989a). These results initially suggested the possible existence of ET receptor subtypes. Two cDNAs were cloned and named ET_A and ET_B receptors and can be distinguished by receptor affinities of the three ET isopeptides (Arai *et al.*, 1990; Sakurai *et al.*, 1990). ET-1 and ET-2 have an equal affinity to ET_A receptors, but ET-3 has less affinity to the ET_A receptor. In contrast, all three isopeptides have nearly identical affinity for the ET_B receptor.

Of the ET peptides, ET-1 is the primary isoform found in the circulation, and most studies would suggest that ET-1 mediates the majority of the cardiovascular and renal effects related to the ET system. Synthesis of the biologically active 21-amino acid ET-1 peptide involves multiple steps. The transcription of the human *edn1* gene encodes roughly 2 kilobase pairs of mRNA, which is translated into a 212-amino acid preproET-1 (Inoue *et al.*, 1989b). Furin-like proteases then cleave preproET-1 to the 38-amino acid precursor, big ET-1. Big ET-1 is released by endothelial cells and can be found in the circulation; however, this peptide has no vasoconstrictor activity or affinity for the ET receptors. The final process of ET-1 synthesis is proteolysis of big ET-1 to ET-1 by endothelin-converting enzyme (ECE) (Masaki *et al.*, 1991). Peptidases, such as neprilysin and deamidase, play a role in enzymatic degradation of ET-1 (Abassi *et al.*, 1993). However, a significant part of ET-1 removal from the circulation occurs through receptor binding and internalization, mainly via ET_B receptors (Loffler *et al.*, 1993).

Physiology of the ET system in the kidney

The entire ET system is highly expressed in the kidney and most abundantly in the renal medulla. Almost every cell type in the kidney produces ET-1 and expresses ET receptors (Kohan *et al.*, 2011b). *In vitro* studies reveal that ET-1 is mainly produced from vascular endothelial cells and tubular epithelial cells. Based on the fact that ET-1 secretion is primarily towards the basal side of cells in culture, it is believed that renal tubular cells most likely release ET-1 mainly towards the interstitial space rather than into the lumen to activate ET_A and ET_B receptors. ET_A receptors are highly expressed in (i) vascular smooth muscle cells of afferent and efferent arterioles; (ii) pericytes associated with vasa recta capillaries; (iii) mesangial cells; and (iv) podocytes, but expression in tubular epithelial cells is fairly low. ET_B receptors are also expressed on vascular smooth muscle cells of afferent and efferent arterioles, but their functional distribution is clearly less than the ET_A receptor and varies along the length of the vascular tree. The activation of ET_A or ET_B receptors on vascular smooth muscle cells increases intracellular Ca^{2+} leading to vasoconstriction. In contrast, ET_B receptors are highly distributed on vascular endothelial cells, vasa recta capillaries, and renal tubular cells, and are in especially high abundance in inner medullary collecting duct (IMCD) cells (Yamamoto and Uemura, 1998). The activation of ET_B receptors on endothelial and tubular cells stimulates nitric oxide (NO) production, leading to vasodilation and natriuresis (Pollock and Pollock, 2008). Thus, ET-1 has disparate actions on the kidney depending on the site of action, vascular or tubular, which each can have profound effects on haemodynamics and fluid and electrolyte transport.

ET-1 in the renal vascular system

The kidney is the most sensitive organ to the vasoconstrictor properties of exogenous ET-1 (Clozel and Clozel, 1989). Intravascular administration of moderate doses of ET-1 increases renal vascular resistance and causes a reduction of glomerular filtration and renal blood flow, which was abolished by ET_A receptor blockade (Brooks *et al.*, 1994; Pollock and Opgenorth, 1994). Higher doses of ET-1 result in ET_B-dependent vasoconstriction as well (Pollock and Opgenorth, 1993). ET-1 has more profound vasoconstrictor effects on the afferent arteriole compared with the efferent arteriole (Loutzenhiser *et al.*, 1990; Takenaka *et al.*, 1993; Inscho *et al.*, 2005; Schildroth *et al.*, 2011). Both ET_A and ET_B receptors can produce vasoconstriction in isolated afferent and efferent arterioles (Edwards *et al.*, 1990; Inscho *et al.*, 2005; Guan and Inscho, 2011; Schildroth *et al.*, 2011) with some reports that ET_B constriction increases along the renal microcirculation (Schildroth *et al.*, 2011). However, activation of ET_B receptors in endothelial cells on the efferent arteriole leads to vasodilation (Inscho *et al.*, 2005; Guan and Inscho, 2011). It is possible that these differences could be due to differential ET receptor distribution in cortical versus juxtamedullary nephrons, but this idea has yet to be explored experimentally.

The vasa recta capillaries provide the majority of blood supply to the renal medulla. These capillaries are derived from efferent arterioles of the juxtamedullary nephron (Pallone *et al.*, 1990). Both ET receptors are located on vasa recta so that the ET system appears to regulate medullary blood flow, which controls the medullary osmotic concentration gradient, Starling forces within the tubular-vascular system, and ultimately sodium and water excretion. Stimulation of ET_A receptors on pericytes reduces capillary diameter and thus medullary blood flow, while ET_B receptor activation on vasa recta endothelium is thought to have an opposite effect through the release of NO (Kohan, 2006). These effects are independent of changes in total renal blood flow or cortical blood flow (Vassileva *et al.*, 2003).

ET-1 in podocytes and mesangial cells

Increasing evidence suggests that activation of the ET system in podocytes and mesangial cells contributes to glomerular dysfunction in a variety of renal diseases, and is a contributing factor towards proteinuria. Mesangial cells release ET-1 under various stimuli, including in response to vasoactive substances, growth factors, and oxidative stress (Sorokin and Kohan, 2003). Podocytes and mesangial cells express mRNA for both ET_A and ET_B receptors, although there is no clear evidence for functional ET_B-mediated effects (Kohan *et al.*, 2011b). ET_A receptor activity promotes actin cytoskeleton remodelling, nephrin shedding and increased expression of pro-inflammatory factors in podocytes (Morigi *et al.*, 2006; Fligny *et al.*, 2011). Similarly, ET-1 induces contraction, proliferation and hypertrophy of mesangial cells as well as collagen deposition through activation of ET_A receptors (Sorokin and Kohan, 2003). In contrast, ET_B receptors alleviate ET_A-dependent mesangial cell contraction via an increase in prostaglandin E_2 and NO production (Sorokin and Kohan, 2003).

ET-1 and renal tubular function

While most renal tubular cells express mRNA for both ET_A and ET_B receptors, the latter predominantly exist in the proximal tubule, thick ascending limb (TAL) and collecting duct (CD) (Yamamoto and Uemura, 1998). The highest expression of ET-1, as well as ET_B receptors, is in the principal cells of the IMCD, a major cell type in the renal medulla. In both males and females, ET-1 inhibits sodium transport or channel activity along the nephron mainly via ET_B receptor activation, leading to increased water and sodium excretion. Moreover, the diuretic and natriuretic effects of ET-1 can be independent of haemodynamic changes (Perico *et al.*, 1991). There are a variety of mechanisms by which ET-1 can inhibit transport. ET-1 suppresses Na^+/K^+ ATPase in proximal tubule and CD cells (Zeidel *et al.*, 1989; Garvin and Sanders, 1991). ET-1 inhibits Cl^- flux in isolated TAL, which can be blocked by an ET_B antagonist (Plato *et al.*, 2000). ET-1 also reduces $Na^+/K^+/2Cl^-$ co-transporter activity in isolated TAL via a NO synthase (NOS) 3 dependent pathway (Herrera *et al.*, 2009). Lastly, ET-1 directly reduces the epithelial Na channel activity via NO and mitogen activated protein kinase pathway in CD (Stricklett *et al.*, 2006; Bugaj *et al.*, 2008).

Genetically modified animal models, which were evaluated using a combination of both male and female mice, reveal the importance of the CD ET system in blood pressure regulation. CD-specific ET-1 and ET_B receptor knockout mice have increased blood pressure while maintained on a normal salt diet (Ahn *et al.*, 2004; Ge *et al.*, 2006). In contrast, CD-specific ET_A receptor knockout mice have comparable blood pressure to genetic controls (Ge *et al.*, 2005). On a high salt diet, CD-specific ET-1 and ET_B receptor knockout mice have further elevations of blood pressure. However, the CD-specific ET-1 knockout mice have an increase in blood pressure roughly twice as large as mice lacking the ET_B receptor in CD after placing the animals on a high salt diet (Ahn *et al.*, 2004; Ge *et al.*, 2006). Importantly, CD $ET_{A/B}$ double knockout mice have a similar blood pressure response as CD ET-1 knockout animal during a high salt diet (Ge *et al.*, 2008), suggesting that ET_B receptors play a key role in sodium excretion and blood pressure control, while ET_A receptors may also contribute blood pressure control in a complex synergistic manner. In each of these CD knockout strains of mice, no differences were noted between male and female mice in terms of blood pressure responses to high salt intake (D. Kohan, pers. comm.).

The ET system in hypertension

Hypertension is the most common disease that affects people around the world and has a unique pathogenesis in men versus women. Premenopausal women have a lower prevalence of high blood pressure than men at the same age. However, the prevalence of high blood pressure in postmenopausal women and men is similar (Lloyd-Jones *et al.*, 2010), suggesting that sex hormones can influence systems that regulate blood pressure. Animal models of hypertension, such as spontaneously hypertensive rats (Reckelhoff *et al.*, 2000; Sullivan *et al.*, 2007), deoxycorticosterone acetate (DOCA) salt (Crofton and Share, 1997; Kawanishi *et al.*, 2007) and chronic angiotensin (Ang) II infusion (Tatchum-Talom *et al.*, 2005; Sampson *et al.*, 2008; Kittikulsuth *et al.*, 2011), also demonstrate that female rats are more resistant to blood pressure elevation compared with males.

As detailed in the previous section, the ET system is a physiologically important regulator of sodium balance and blood pressure. Pharmacological blockade and genetic modification of the ET_B receptor in both male and female rodent models increase blood pressure, especially when on a high salt diet. Our laboratory observed that chronic blockade of the ET_B receptor increases blood pressure in male and female rats on a normal salt diet, which is much higher on a high salt diet (Pollock and Pollock, 2001; Sullivan *et al.*, 2006). Gariepy *et al.* reported that rats with a genetically dysfunctional ET_B receptor had a higher blood pressure than control groups under baseline conditions. When ET_B-deficient rats received a high salt diet, blood pressure was dramatically increased, an effect blocked by amiloride (Gariepy *et al.*, 2000). As previously mentioned, CD ET-1 and ET_B receptor knockout mice also develop high blood pressure the magnitude of which is dependent upon the level of salt intake (Ahn *et al.*, 2004; Ge *et al.*, 2006). These data support that the impairment of ET_B receptor function leads to salt-sensitive hypertension.

Accumulating evidence over the past 10 years also demonstrates that an interaction between the ET system and NO pathway regulates sodium balance. Nakano *et al.* showed that stimulation of the ET_B receptor in the renal medulla of male rats with an ET_B receptor agonist, sarafotoxin 6c (S6c), increased water and sodium excretion independent of changes in blood pressure and medullary blood flow. This effect was absent in male ET_B receptor deficient rats. The authors also showed that ET_B induced diuresis and natriuresis is NOS1 dependent [Figure 1; (Nakano *et al.*, 2008)]. Schneider *et al.* demonstrated that the differences in blood pressure between CD ET-1 knockout and control mice were abolished by administration of NOS inhibitor (Schneider *et al.*, 2008).

Sex differences in the ET system in the kidney

The renal ET system has been shown to account for sex differences in sodium regulation and blood pressure control.

More specifically, the role of ET_B receptors in the different blood pressure responses to pharmacological or environmental manipulations, including chronic mineralocorticoid (DOCA) treatment or a high salt diet, between males and females has been explored using ET_B receptor deficient rats. Taylor *et al*. showed that, on a normal salt diet, male rats lacking functional ET_B receptors had higher blood pressure than female counterparts, while plasma ET-1 was comparable in both groups. They also reported that renal medullary NOS activity was higher in female ET_B-deficient rats compared with males while given a normal salt diet (Taylor *et al*., 2003). These data suggest that ET_A-dependent blood pressure increases are more dominant in male rats and renal NOS may act as a protective mechanism in female rats. In contrast, female ET_B-deficient rats developed more severe hypertension compared with males in response to a high salt diet. The difference in blood pressure elevation corresponds with a marked reduction of urinary ET-1 excretion (Taylor *et al*., 2003). Sullivan *et al*. further demonstrated that hypertension in rats lacking functional ET_B receptors in both sexes during high salt feeding was due in part to the production of reactive oxygen species. However, blood pressure elevation in female ET_B-deficient rats was more dependent on the increase in oxidative stress because administration of tempol, a scavenger of reactive oxygen species, reduced blood pressure to similar levels between male and female rats (Sullivan *et al*., 2006). These data emphasize that ET_B receptors provide a protective mechanism against blood pressure elevation in female rats. The question of whether ET_B receptor function is different between males and females has yet to be explored in mice or humans.

ET_A receptor activation contributes to DOCA-salt-induced blood pressure elevation, while renal ET_B receptors have a protective role in the DOCA-salt model. ET_B receptor blockade further increased blood pressure in DOCA-salt model, albeit not to the same extent as in normotensive animals. Moreover, renal medullary ET_B receptor binding was increased in this model (Pollock *et al*., 2000). Sex hormones play a role in DOCA-salt-induced hypertension. Male rats become more hypertensive compared with female rats following DOCA-salt treatment. Ovariectomy further elevates blood pressure in DOCA-salt treated female rats, while castration of males attenuates hypertension (Crofton and Share, 1997; Montezano *et al*., 2005; Kawanishi *et al*., 2007). Montezano *et al*. demonstrated that ET-1 mRNA in the kidney of male rats was increased compared with females in the DOCA-salt model. Ovariectomized female DOCA-salt rats had increased renal ET-1 mRNA expression to a similar level as male DOCA-salt rats. The authors also showed that male DOCA-salt rats exhibited more severe renal damage than females; ET_A receptor blockade alleviated much of the renal damage in both sexes (Montezano *et al*., 2005). While it was reported earlier that female ET_B-deficient rats have higher blood pressure and oxidative stress within the kidney in response to a high salt diet, Kawanishi *et al*. reported that female ET_B-deficient rats had a similar increase in blood pressure as male ET_B-deficient rats in the DOCA-salt model. However, male ET_B-deficient rats produced more oxidative stress in aortic tissue compared with females (Kawanishi *et al*., 2007), suggesting that the ET_B receptor provides relative protection against DOCA-salt hypertension in both male and female rats, yet its influence on pro-hypertensive mechanisms may be different between the sexes. Thus, the nature of how ET_B receptors contribute to sex differences in blood pressure regulation through oxidative stress in the kidney in DOCA-salt rats remains somewhat speculative.

Our laboratory has recently provided evidence that renal medullary ET_A receptors also contribute to sex differences in blood pressure regulation. As described above, Nakano *et al*. demonstrated that stimulation of the ET_B receptor in the renal medulla by S6c increases water and sodium excretion in both male and female wild-type control rats. The authors also infused ET-1 into the renal medulla of male and female ET_B-deficient rats or genetic controls. They found that both control and ET_B-deficient female rats had increased water and sodium excretion, while male rats did not respond to ET-1 in terms of a diuresis or natriuresis. These different responses to ET-1 infusion could be explained by the reduction of medullary blood flow in male rats that was not seen in female rats. They further showed that ET-1-induced water and sodium excretion in female ET_B-deficient rats is mediated by ET_A-dependent and NOS1-dependent mechanisms because administration of an ET_A receptor antagonist or a selective NOS1 inhibitor into renal medulla could abolish the response to intramedullary ET-1 infusion. Moreover, ovariectomized female ET_B-deficient rats had an attenuated response to ET-1 infusion that was associated with a reduction in medullary blood flow much like male rats (Nakano and Pollock, 2009). This study emphasizes the relationship between renal medullary haemodynamic and tubular function in controlling sodium excretion (Figure 1). Moreover, they demonstrate the importance of sex hormones on renal medullary ET-1-mediated responses in males versus females. The question of whether tubular ET_A receptor-dependent inhibition of salt and water re-absorption occurs in male rats, but is masked by reductions in medullary blood flow, needs to be investigated.

We have also applied the intramedullary infusion technique to examine the ET receptor function in both male and female rats made hypertensive by chronic infusion of Ang II (Kittikulsuth *et al*., 2011). We found that male Ang II hypertensive rats did not respond to S6c with any changes in water and sodium excretion, which was associated with a reduction of ET_B receptor binding sites. In contrast, female Ang II hypertensive rats, which expressed comparable ET_B binding sites to the female normotensive group, have preserved responses to ET_B agonist-induced diuresis and natriuresis. Nonetheless, ET_B-dependent excretory effects in female Ang II hypertensive rats were reduced when compared with female normotensive rats. We further examined the role of ET_A-mediated response in renal medulla of female Ang II hypertension because male rats do not display ET-1-dependent water and sodium excretion in our model (Nakano and Pollock, 2009). Renal medullary ET-1 infusion stimulated water and sodium excretion in female rats following chronic Ang II infusion, which is partially blunted by ET_A receptor blockade (Kittikulsuth *et al*., 2011). These data suggest that ET_A-mediated diuresis and natriuresis is maintained to some degree in female Ang II hypertensive rats. The hypothetical scheme for how renal ET_A and ET_B receptors differ under circumstances of Ang II-dependent hypertension is depicted in Figure 2.

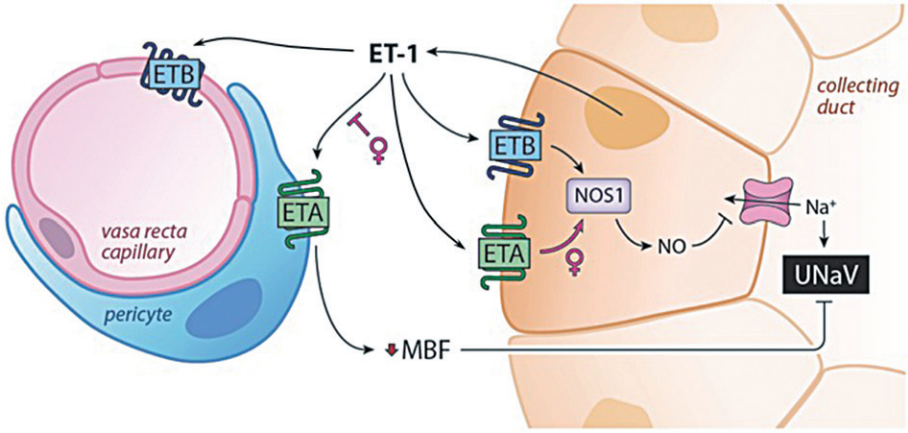

Figure 1

Hypothetical scheme depicting the sex difference that occurs in Ang II-dependent hypertension. In both males and females, Ang II contributes to ET_B receptor dysfunction that leads to reduced fluid and electrolyte excretion. However, in females, the additional ET_A-dependent pro-natriuretic mechanisms may account for reduced hypertension in the female. MBF, medullary blood flow.

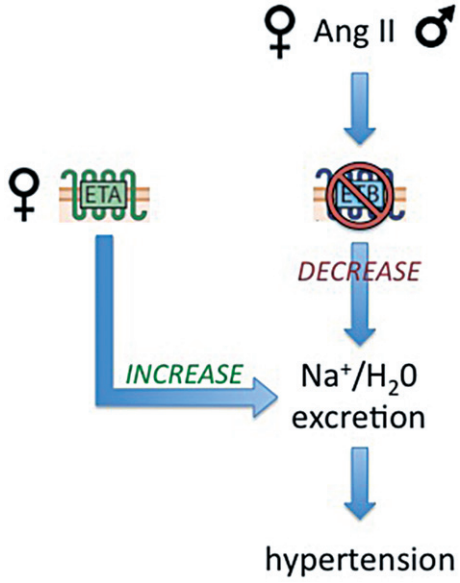

Figure 2

Sex differences in the physiological function of the renal medullary endothelin system in control of sodium and water excretion.

The ET system in renal injury

There is growing evidence to suggest that males and females have a different susceptibility to renal injury. In clinical settings, women have a slower regression of renal function with age compared with men (Berg, 2006). Moreover, women are also less likely to develop various renal diseases, including chronic kidney disease or polycystic kidney disease, compared with men (Neugarten *et al.*, 2000). There is considerable evidence that the ET system plays a key role in pathophysiology of kidney disease including the observation that over-expression of human ET-1 in mice results in development of renal cysts and fibrosis (Hocher *et al.*, 1997). Moreover, ET antagonists ameliorate the progression of chronic kidney disease in animal models (Neuhofer and Pittrow, 2009). However, little is known about how the ET system participates in the sexual dimorphism associated with renal injury.

Polycystic kidney disease

Autosomal-dominant polycystic kidney disease (ADPKD) is the most common genetic kidney disease characterized by the slow development of large fluid-filled cysts in the kidneys and other organs, including liver. Approximately 50% of ADPKD will progress to end-stage renal disease (ESRD) (Torres and Harris, 2009). Men have a more progressive form of ADPKD compared with women (Neugarten *et al.*, 2000; Torres and Harris, 2009). Han:SPRD, a rat model of ADPKD, shows similar sex differences in the progressive loss of renal function in which females develop renal lesions at an older age compared with males (Gretz *et al.*, 1995). Stringer *et al.* reported additional evidence that ET-1 may play a role in the sexual dimorphism associated with renal damage in Han:SPRD rats (Stringer *et al.*, 2005). Compared with females, male Han:SPRD rats have dramatically increased proteinuria, reduced renal function and higher cyst numbers in the kidney, while female cystic rats do not have an increase in proteinuria or differences in renal function compared with non-cystic females. Moreover, ovariectomy led to a significant impairment of renal function. The authors also reported that kidneys from cystic males had markedly increased ET-1 levels compared with non-cystic controls. Renal ET-1 content was also increased in female cystic rats, but to a lesser degree compared with male rats. Furthermore, ovariectomized cystic rats had comparable amounts of renal ET-1 as male rats. These data suggest that female sex hormones regulate the renal ET-1 system in the pathogenesis of polycystic kidney disease in Han:SPRD rats.

Acute kidney injury

Ischaemia/reperfusion (I/R) injury, a major cause of acute kidney injury (AKI), is common in patients undergoing cardiovascular surgery or kidney transplantation. Many animal studies show that males are more vulnerable than females to the development of renal I/R injury. Park *et al*. demonstrated that male mice had impaired renal function after 30 min bilateral renal ischaemia, while females had relatively preserved renal function (Park *et al*., 2004). Moreover, male mice had a higher mortality rate after 60 min of renal ischaemia (Park *et al*., 2004). Castration had a protective effect against ischaemic renal damage (Park *et al*., 2004). Similarly, uninephrectomized male rats developed a severe AKI with an 8% survival rate within 2–4 days following 50 min of ischaemia, while in contrast, females had a 75% survival rate. Moreover, orchidectomy attenuated renal damage and mortality such that males were then comparable to females (Muller *et al*., 2002). Muller *et al*. further reported that renal ET-1 mRNA expression was substantially higher in male rats, while females had no change in ET-1 expression 2 h following 50 min renal ischaemia. Interestingly, pretreatment with an ET_A receptor antagonist for 7 days improved the survival rate in male rats, while ET_A receptor blockade actually increased mortality in females (Muller *et al*., 2002). These data clearly support the hypothesis that ET_A receptors are involved in the severity of renal damage following renal ischaemia, which is more unfavourable in males. However, ET_A receptors surprisingly provide a protective effect in females and will need to be investigated in future studies.

Clinical studies of ET receptor antagonists in hypertension and renal disease

Selective ET_A antagonists, including atrasentan, avosentan, darusentan and sitaxsentan, have been investigated in clinical studies as a treatment for hypertension and renal disease. ET_A antagonists have shown an effectiveness to reduce blood pressure in patients with refractory hypertension (Weber *et al*., 2009), as well as chronic kidney disease (Mann *et al*., 2010). Furthermore, ET_A antagonist treatment diminishes albuminuria and proteinuria in diabetes and chronic kidney disease patients (Dhaun *et al*., 2009; Wenzel *et al*., 2009; Mann *et al*., 2010; Kohan *et al*., 2011a). However, the major adverse effect with the use of ET_A antagonists in those patients is the dose-dependent incidence of oedema. For this reason, one major clinical trial was prematurely terminated (Wenzel *et al*., 2009). The possible mechanisms of fluid overload are that the doses of antagonists may not have maintained selectivity for ET_A receptors and inhibit ET_B receptors, which leads to impaired water and sodium excretion. It is also possible that the ET_A receptor contributes to salt and water excretion in humans, especially when ET_B receptor function may be impaired, as has been shown in animal model studies (Kohan *et al*., 2011b). In addition, inhibition of ET_A receptors may cause preferential arteriolar dilation and increase capillary hydrostatic pressure, which may also facilitate fluid retention (Kohan *et al*., 2011a).

As mentioned above, preclinical studies have shown that ET_B as well as ET_A, receptor activities may promote blood pressure lowering and renal protective effects, such that ET_A antagonists may be less efficacious in women compared with men. Currently, there are very little data provided on the therapeutic effects or adverse events from clinical trials separately between men and women after ET_A antagonist treatment. In general, men have been the major population in almost all clinical studies of ET_A antagonists. One study did report that men and women with resistant hypertension had a similar decrease in systolic blood pressure after darusentan treatment (Weber *et al*., 2009). However, the investigators did not further analyse other parameters, such as the incidence of oedema, with regard to sex. Thus, the questions whether women and men respond differently to ET_A antagonists need to be investigated.

Genetic polymorphism of the ET system in hypertension and kidney disease

Several single nucleotide polymorphisms (SNPs) of the genes for ET-1 (EDN1), ECE, ET_A (EDNRA) and ET_B (EDNRB) receptors have been found and reported to be associated with hypertension and renal injury in humans. Most studies use a candidate gene approach to examine the association between hypertension or renal damage and the allele frequency of polymorphic ET gene markers in patients and healthy controls (Binder, 2007). A few studies report the sex differences of the frequency or association of gene variants with these diseases. Mainly, the polymorphisms of ECE and EDNRA genes are more related to the progression of high blood pressure or kidney disease in women in comparison to men.

G-to-T transversion at codon 198 in exon 5 leads to a change in amino acid residue 198 from lysine to asparagine (Lys198Asn or K198N) in the EDN1 gene and was reported to be associated with blood pressure elevation in both the male and female overweight population. The T198 allele in obese subjects had higher systolic or diastolic blood pressure than those with GG genotype (Tiret *et al*., 1999; Asai *et al*., 2001). Interestingly, the polymorphisms of ECE-1b and EDNRA have been shown to be associated with blood pressure levels in women. The two common variants of C-338A and T-839G (-338A and -839G alleles) of 5′ untranslated regions of the ECE-1b gene showed a significant increase in blood pressure in females (Funke-Kaiser *et al*., 2003). The authors also showed that the -338A allele was associated with an increase in ECE-1b promotor activity (Funke-Kaiser *et al*., 2003), which may lead to an increase in ET-1 production. However, this study did not measure plasma ET-1 levels or provide any data to suggest increased ET production in their study population. Furthermore, female AA homozygotes of 5′ untranslated regions of the EDNRA gene had significant higher systolic and diastolic blood pressure compared with other phenotypes. Several polymorphisms of EDNRB had been reported; however, they were not associated with hypertension (Nicaud *et al*., 1999; Caprioli *et al*., 2008).

The influence of EDN1, ECE and EDNRA gene polymorphisms has been shown to be associated with the early onset

of ADPKD and IgA nephropathy. In a Czech population, a K198N (G/T substitution at codon 198) in exon 5, an insertion of adenine (3A/4A) or a T-1370G in the promotor of EDN1 gene had been found to distribute equally in male and female ADPKD patients. Each type of SNP variants did not correlate to an onset or progression of ADPKD or IgA nephropathy. However, the combination of different haplotypes (4A allele and 198K allele or 4A allele, 198G and -1370G) was reported to be significantly associated to the early onset or progression of end-stage renal disease in ADPKD and IgA nephropathy (Reiterova *et al.*, 2006a; Maixnerova *et al.*, 2007). Moreover, the C-338A polymorphism in the ECE gene had been found in ADPKD patients. AA homozygocity in the ECE gene had a tendency towards a lower age of ESRD compared with other genotypes (Reiterova *et al.*, 2006b). A CT polymorphism in exon 8 of EDNRA gene has been shown to be associated with the progression of ADPKD. Women with ADPKD having the CT genotype developed ESRD an average of 4 years earlier compared with those with the CC allele. In contrast, there was no influence of CT polymorphism on the progression of ADPKD in males (Reiterova *et al.*, 2007). Because all studies were limited to the Czech population, the question whether the association between the genetic variants of the ET system and kidney diseases can be observed in a broader range of populations needs to be confirmed.

Conclusions

In conclusion, it is now clearly established that the ET system plays a role in modulating kidney function and blood pressure control. Renal ET receptors function somewhat differently between males and females and appear to be regulated by sex steroids. ET_A receptor activation produces unfavourable effects in male kidneys, including renal medullary vasoconstriction and renal injury. In contrast, females are relatively protected against high blood pressure and kidney damage by virtue of increased ET_B receptor function and perhaps reduced ET_A-dependent haemodynamic effects. Moreover, ET_A receptors may also serve as a defence mechanism against renal damage in females through yet unidentified mechanisms. Understanding of the underlying mechanism of how the ET system is involved in phenotypic dimorphism may lead to the better treatment in kidney-related diseases.

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Conflict of interest

None.

References

Abassi ZA, Golomb E, Bridenbaugh R, Keiser HR (1993). Metabolism of endothelin-1 and big endothelin-1 by recombinant neutral endopeptidase EC.3.4.24.11. Br J Pharmacol 109: 1024–1028.

Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK *et al.* (2004). Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. J Clin Invest 114: 504–511.

Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S (1990). Cloning and expression of a cDNA encoding an endothelin receptor. Nature 348: 730–732.

Asai T, Ohkubo T, Katsuya T, Higaki J, Fu Y, Fukuda M *et al.* (2001). Endothelin-1 gene variant associates with blood pressure in obese Japanese subjects: the Ohasama Study. Hypertension 38: 1321–1324.

Berg UB (2006). Differences in decline in GFR with age between males and females. Reference data on clearances of inulin and PAH in potential kidney donors. Nephrol Dial Transplant 21: 2577–2582.

Binder A (2007). A review of the genetics of essential hypertension. Curr Opin Cardiol 22: 176–184.

Brooks DP, Depalma PD, Pullen M, Nambi P (1994). Characterization of canine renal endothelin receptor subtypes and their function. J Pharmacol Exp Ther 268: 1091–1097.

Bugaj V, Pochynyuk O, Mironova E, Vandewalle A, Medina JL, Stockand JD (2008). Regulation of the epithelial Na+ channel by endothelin-1 in rat collecting duct. Am J Physiol Renal Physiol 295: F1063–F1070.

Caprioli J, Mele C, Mossali C, Gallizioli L, Giacchetti G, Noris M *et al.* (2008). Polymorphisms of EDNRB, ATG, and ACE genes in salt-sensitive hypertension. Can J Physiol Pharmacol 86: 505–510.

Clozel M, Clozel JP (1989). Effects of endothelin on regional blood flows in squirrel monkeys. J Pharmacol Exp Ther 250: 1125–1131.

Crofton JT, Share L (1997). Gonadal hormones modulate deoxycorticosterone-salt hypertension in male and female rats. Hypertension 29: 494–499.

Dhaun N, Macintyre IM, Melville V, Lilitkarntakul P, Johnston NR, Goddard J *et al.* (2009). Blood pressure-independent reduction in proteinuria and arterial stiffness after acute endothelin-a receptor antagonism in chronic kidney disease. Hypertension 54: 113–119.

Edwards RM, Trizna W, Ohlstein EH (1990). Renal microvascular effects of endothelin. Am J Physiol 259: F217–F221.

Fligny C, Barton M, Tharaux PL (2011). Endothelin and podocyte injury in chronic kidney disease. Contrib Nephrol 172: 120–138.

Funke-Kaiser H, Reichenberger F, Kopke K, Herrmann SM, Pfeifer J, Orzechowski HD *et al.* (2003). Differential binding of transcription factor E2F-2 to the endothelin-converting enzyme-1b promoter affects blood pressure regulation. Hum Mol Genet 12: 423–433.

Gariepy CE, Ohuchi T, Williams SC, Richardson JA, Yanagisawa M (2000). Salt-sensitive hypertension in endothelin-B receptor-deficient rats. J Clin Invest 105: 925–933.

Garvin J, Sanders K (1991). Endothelin inhibits fluid and bicarbonate transport in part by reducing Na+/K+ ATPase activity in the rat proximal straight tubule. J Am Soc Nephrol 2: 976–982.

Ge Y, Stricklett PK, Hughes AK, Yanagisawa M, Kohan DE (2005). Collecting duct-specific knockout of the endothelin A receptor alters renal vasopressin responsiveness, but not sodium excretion or blood pressure. Am J Physiol Renal Physiol 289: F692–F698.

Ge Y, Bagnall A, Stricklett PK, Strait K, Webb DJ, Kotelevtsev Y *et al.* (2006). Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention. Am J Physiol Renal Physiol 291: F1274–F1280.

Ge Y, Bagnall A, Stricklett PK, Webb D, Kotelevtsev Y, Kohan DE (2008). Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. Am J Physiol Renal Physiol 295: F1635–F1640.

Gretz N, Ceccherini I, Kranzlin B, Kloting I, Devoto M, Rohmeiss P *et al.* (1995). Gender-dependent disease severity in autosomal polycystic kidney disease of rats. Kidney Int 48: 496–500.

Guan Z, Inscho EW (2011). Endothelin and the renal vasculature. Contrib Nephrol 172: 35–49.

Herrera M, Hong NJ, Ortiz PA, Garvin JL (2009). Endothelin-1 inhibits thick ascending limb transport via Akt-stimulated nitric oxide production. J Biol Chem 284: 1454–1460.

Hickey KA, Rubanyi G, Paul RJ, Highsmith RF (1985). Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. Am J Physiol 248: C550–C556.

Hocher B, Thone-Reineke C, Rohmeiss P, Schmager F, Slowinski T, Burst V *et al.* (1997). Endothelin-1 transgenic mice develop glomerulosclerosis, interstitial fibrosis, and renal cysts but not hypertension. J Clin Invest 99: 1380–1389.

Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K *et al.* (1989a). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA 86: 2863–2867.

Inoue A, Yanagisawa M, Takuwa Y, Mitsui Y, Kobayashi M, Masaki T (1989b). The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. J Biol Chem 264: 14954–14959.

Inscho EW, Imig JD, Cook AK, Pollock DM (2005). ETA and ETB receptors differentially modulate afferent and efferent arteriolar responses to endothelin. Br J Pharmacol 146: 1019–1026.

Kawanishi H, Hasegawa Y, Nakano D, Ohkita M, Takaoka M, Ohno Y *et al.* (2007). Involvement of the endothelin ET(B) receptor in gender differences in deoxycorticosterone acetate-salt-induced hypertension. Clin Exp Pharmacol Physiol 34: 280–285.

Kittikulsuth W, Pollock JS, Pollock DM (2011). Sex differences in renal medullary endothelin receptor function in angiotensin II hypertensive rats. Hypertension 58: 212–218.

Kohan DE (2006). The renal medullary endothelin system in control of sodium and water excretion and systemic blood pressure. Curr Opin Nephrol Hypertens 15: 34–40.

Kohan DE, Pritchett Y, Molitch M, Wen S, Garimella T, Audhya P *et al.* (2011a). Addition of atrasentan to renin-angiotensin system blockade reduces albuminuria in diabetic nephropathy. J Am Soc Nephrol 22: 763–772.

Kohan DE, Rossi NF, Inscho EW, Pollock DM (2011b). Regulation of blood pressure and salt homeostasis by endothelin. Physiol Rev 91: 1–77.

Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G *et al.* (2010). Heart disease and stroke statistics – 2010 update: a report from the American Heart Association. Circulation 121: e46–e215.

Loffler BM, Breu V, Clozel M (1993). Effect of different endothelin receptor antagonists and of the novel non-peptide antagonist Ro 46-2005 on endothelin levels in rat plasma. FEBS Lett 333: 108–110.

Loutzenhiser R, Epstein M, Hayashi K, Horton C (1990). Direct visualization of effects of endothelin on the renal microvasculature. Am J Physiol 258: F61–F68.

Maixnerova D, Merta M, Reiterova J, Stekrova J, Rysava R, Obeidova H *et al.* (2007). The influence of three endothelin-1 polymorphisms on the progression of IgA nephropathy. Folia Biol 53: 27–32.

Mann JF, Green D, Jamerson K, Ruilope LM, Kuranoff SJ, Littke T *et al.* (2010). Avosentan for overt diabetic nephropathy. J Am Soc Nephrol 21: 527–535.

Masaki T, Kimura S, Yanagisawa M, Goto K (1991). Molecular and cellular mechanism of endothelin regulation. Implications for vascular function. Circulation 84: 1457–1468.

Montezano AC, Callera GE, Mota AL, Fortes ZB, Nigro D, Carvalho MH *et al.* (2005). Endothelin-1 contributes to the sexual differences in renal damage in DOCA-salt rats. Peptides 26: 1454–1462.

Morigi M, Buelli S, Zanchi C, Longaretti L, Macconi D, Benigni A *et al.* (2006). Shigatoxin-induced endothelin-1 expression in cultured podocytes autocrinally mediates actin remodeling. Am J Pathol 169: 1965–1975.

Muller V, Losonczy G, Heemann U, Vannay A, Fekete A, Reusz G *et al.* (2002). Sexual dimorphism in renal ischemia-reperfusion injury in rats: possible role of endothelin. Kidney Int 62: 1364–1371.

Nakano D, Pollock DM (2009). Contribution of endothelin A receptors in endothelin 1-dependent natriuresis in female rats. Hypertension 53: 324–330.

Nakano D, Pollock JS, Pollock DM (2008). Renal medullary ETB receptors produce diuresis and natriuresis via NOS1. Am J Physiol Renal Physiol 294: F1205–F1211.

Neugarten J, Acharya A, Silbiger SR (2000). Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. J Am Soc Nephrol 11: 319–329.

Neuhofer W, Pittrow D (2009). Endothelin receptor selectivity in chronic kidney disease: rationale and review of recent evidence. Eur J Clin Invest 39 (Suppl. 2): 50–67.

Nicaud V, Poirier O, Behague I, Herrmann SM, Mallet C, Troesch A *et al.* (1999). Polymorphisms of the endothelin-A and -B receptor genes in relation to blood pressure and myocardial infarction: the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) Study. Am J Hypertens 12: 304–310.

Pallone TL, Robertson CR, Jamison RL (1990). Renal medullary microcirculation. Physiol Rev 70: 885–920.

Park KM, Kim JI, Ahn Y, Bonventre AJ, Bonventre JV (2004). Testosterone is responsible for enhanced susceptibility of males to ischemic renal injury. J Biol Chem 279: 52282–52292.

Perico N, Cornejo RP, Benigni A, Malanchini B, Ladny JR, Remuzzi G (1991). Endothelin induces diuresis and natriuresis in the rat by acting on proximal tubular cells through a mechanism mediated by lipoxygenase products. J Am Soc Nephrol 2: 57–69.

Plato CF, Pollock DM, Garvin JL (2000). Endothelin inhibits thick ascending limb chloride flux via ET(B) receptor-mediated NO release. Am J Physiol Renal Physiol 279: F326–F333.

Pollock DM, Opgenorth TJ (1993). Evidence for endothelin-induced renal vasoconstriction independent of ETA receptor activation. Am J Physiol 264: R222–R226.

Pollock DM, Opgenorth TJ (1994). ETA receptor-mediated responses to endothelin-1 and big endothelin-1 in the rat kidney. Br J Pharmacol 111: 729–732.

Pollock DM, Pollock JS (2001). Evidence for endothelin involvement in the response to high salt. Am J Physiol Renal Physiol 281: F144–F150.

Pollock DM, Allcock GH, Krishnan A, Dayton BD, Pollock JS (2000). Upregulation of endothelin B receptors in kidneys of DOCA-salt hypertensive rats. Am J Physiol Renal Physiol 278: F279–F286.

Pollock JS, Pollock DM (2008). Endothelin and NOS1/nitric oxide signaling and regulation of sodium homeostasis. Curr Opin Nephrol Hypertens 17: 70–75.

Reckelhoff JF, Zhang H, Srivastava K (2000). Gender differences in development of hypertension in spontaneously hypertensive rats: role of the renin-angiotensin system. Hypertension 35: 480–483.

Reiterova J, Merta M, Stekrova J, Cabartova Z, Cibulka R, Maixnerova D *et al*. (2006a). Influence of endothelin-1 gene polymorphisms on the progression of autosomal dominant polycystic kidney disease. Kidney Blood Press Res 29: 182–188.

Reiterova J, Merta M, Stekrova J, Tesar V, Kmentova D, Rihova Z *et al*. (2006b). The influence of the endothelin-converting enzyme-1 gene polymorphism on the progression of autosomal dominant polycystic kidney disease. Ren Fail 28: 21–24.

Reiterova J, Merta M, Stekrova J, Maixnerova D, Obeidova H, Kebrdlova V *et al*. (2007). The influence of endothelin-A receptor gene polymorphism on the progression of autosomal dominant polycystic kidney disease and IgA nephropathy. Folia Biol 53: 134–137.

Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K *et al*. (1990). Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 348: 732–735.

Sampson AK, Moritz KM, Jones ES, Flower RL, Widdop RE, Denton KM (2008). Enhanced angiotensin II type 2 receptor mechanisms mediate decreases in arterial pressure attributable to chronic low-dose angiotensin II in female rats. Hypertension 52: 666–671.

Schildroth J, Rettig-Zimmermann J, Kalk P, Steege A, Fahling M, Sendeski M *et al*. (2011). Endothelin type A and B receptors in the control of afferent and efferent arterioles in mice. Nephrol Dial Transplant 26: 779–789.

Schneider MP, Ge Y, Pollock DM, Pollock JS, Kohan DE (2008). Collecting duct-derived endothelin regulates arterial pressure and Na excretion via nitric oxide. Hypertension 51: 1605–1610.

Sokolovsky M (1992). Structure-function relationships of endothelins, sarafotoxins, and their receptor subtypes. J Neurochem 59: 809–821.

Sorokin A, Kohan DE (2003). Physiology and pathology of endothelin-1 in renal mesangium. Am J Physiol Renal Physiol 285: F579–F589.

Stricklett PK, Hughes AK, Kohan DE (2006). Endothelin-1 stimulates NO production and inhibits cAMP accumulation in rat inner medullary collecting duct through independent pathways. Am J Physiol Renal Physiol 290: F1315–F1319.

Stringer KD, Komers R, Osman SA, Oyama TT, Lindsley JN, Anderson S (2005). Gender hormones and the progression of experimental polycystic kidney disease. Kidney Int 68: 1729–1739.

Sullivan JC, Pollock JS, Pollock DM (2006). Superoxide-dependent hypertension in male and female endothelin B receptor-deficient rats. Exp Biol Med (Maywood) 231: 818–823.

Sullivan JC, Semprun-Prieto L, Boesen EI, Pollock DM, Pollock JS (2007). Sex and sex hormones influence the development of albuminuria and renal macrophage infiltration in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 293: R1573–R1579.

Takenaka T, Forster H, Epstein M (1993). Protein kinase C and calcium channel activation as determinants of renal vasoconstriction by angiotensin II and endothelin. Circ Res 73: 743–750.

Tatchum-Talom R, Eyster KM, Martin DS (2005). Sexual dimorphism in angiotensin II-induced hypertension and vascular alterations. Can J Physiol Pharmacol 83: 413–422.

Taylor TA, Gariepy CE, Pollock DM, Pollock JS (2003). Gender differences in ET and NOS systems in ETB receptor-deficient rats: effect of a high salt diet. Hypertension 41: 657–662.

Tiret L, Poirier O, Hallet V, Mcdonagh TA, Morrison C, Mcmurray JJ *et al*. (1999). The Lys198Asn polymorphism in the endothelin-1 gene is associated with blood pressure in overweight people. Hypertension 33: 1169–1174.

Torres VE, Harris PC (2009). Autosomal dominant polycystic kidney disease: the last 3 years. Kidney Int 76: 149–168.

Tostes RC, Fortes ZB, Callera GE, Montezano AC, Touyz RM, Webb RC *et al*. (2008). Endothelin, sex and hypertension. Clin Sci (Lond) 114: 85–97.

Vassileva I, Mountain C, Pollock DM (2003). Functional role of ETB receptors in the renal medulla. Hypertension 41: 1359–1363.

Weber MA, Black H, Bakris G, Krum H, Linas S, Weiss R *et al*. (2009). A selective endothelin-receptor antagonist to reduce blood pressure in patients with treatment-resistant hypertension: a randomised, double-blind, placebo-controlled trial. Lancet 374: 1423–1431.

Wenzel RR, Littke T, Kuranoff S, Jurgens C, Bruck H, Ritz E *et al*. (2009). Avosentan reduces albumin excretion in diabetics with macroalbuminuria. J Am Soc Nephrol 20: 655–664.

Yamamoto T, Uemura H (1998). Distribution of endothelin-B receptor-like immunoreactivity in rat brain, kidney, and pancreas. J Cardiovasc Pharmacol 31 (Suppl. 1): S207–S211.

Yanagisawa M, Inoue A, Ishikawa T, Kasuya Y, Kimura S, Kumagaye S *et al*. (1988a). Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. Proc Natl Acad Sci USA 85: 6964–6967.

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y *et al*. (1988b). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332: 411–415.

Zeidel ML, Brady HR, Kone BC, Gullans SR, Brenner BM (1989). Endothelin, a peptide inhibitor of Na(+)-K(+)-ATPase in intact renaltubular epithelial cells. Am J Physiol 257: C1101–C1107.