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PERSPECTIVES IN CLINICAL NEPHROLOGY

Endothelin in renal pathophysiology: From experimental to therapeutic application

Endothelin was discovered as recently as 1988 as a potent vasoconstrictor peptide [1]. This discovery has opened up a new proliferative field in biomedical research. Increased expression of endothelin in various renal diseases, the injurious actions of endothelin on renal cells *in vitro*, and the beneficial effects of specific blockers of endothelin in experimental renal disease have created a very attractive pathophysiological paradigm in renal medicine, as was reviewed in this journal previously [2–6]. However, at the same time, renal disease is a multifactorial process, and only little information is available on interactions of endothelin with other pathophysiological factors in the kidney. Furthermore, it is unclear whether the experiments with endothelin receptor blockers also prevent structural damage, and whether results of such experiments can be extrapolated to the human situation. The aim of the present review is to place the endothelin concept, as it was introduced in this journal, in a clinical perspective. In the first part we will briefly discuss the current status of this endothelin concept, and in the second part we will discuss its caveats and clinical applicability.

Rationale for the endothelin concept

Endothelin-system in the kidney

The endothelins encompass a family of three isopeptides, which are all cleaved from proendothelins (Big ET's) by an endothelin converting enzyme [1, 7]. The kidney is an important organ for endothelin synthesis. The most important isopeptide is endothelin-1 (ET-1), which is produced by endothelial, mesangial, glomerular epithelial and medullary collecting duct cells [8–11]. Although studies in rats and cell cultures also demonstrated ET-3 production in glomerular epithelial cells and medullary collecting duct cells [8, 10], ET-3 production could not be demonstrated in the human kidney [12]. In normal kidneys ET-1 immunostaining is confined to the endothelium of the intrarenal blood vessels and the glomerulus [12]. ET-1 synthesis is tightly regulated at the transcriptional level. Importantly, various humoral factors involved in glomerular injury, such as thrombin, hypoxia, transforming growth factor-β and angiotensin II can stimulate ET-1 synthesis [1, 13, 14]. ET-1 interacts with type A (ETa) and type B (ETb) receptors. In the human kidney the ETb receptor predominates over the ETa receptor, the ETa receptor being localized in the vasculature and the ETb receptor in renal tubuli and medulla [15, 16]. ET-1 forms very strong ligand-receptor complexes that are rapidly internalized and inactivated [17], resulting in a very short half-life of circulating ET-1 [18, 19]. It is therefore generally believed that ET-1 exerts its actions in the kidney mainly as an autocrine/paracrine factor, which is also suggested by the fact that sites of endothelin-synthesis and endothelin-receptors are closely linked [12]. Despite its short circulating half-life the biological effects of ET-1 usually are sustained for a long period of time. Receptor internalization followed by externalization of new or recycled receptors may contribute to these prolonged biological effects [20, 21]. Multiple intracellular signal transduction systems have been linked to the endothelin receptors (Fig. 1) [22]. The most important one is probably activation of phospholipase C and the inositol-phosphate pathway leading to sustained elevations of intracellular calcium. It has been suggested that the plateau phase of the intracellular calcium response may also play a role in the prolonged responses to endothelin [23]. The putative actions of ET-1 in the kidney include stimulation of renal cell growth [24–26], cell differentiation during organogenesis [27], vasoconstriction [28–30], and inhibition of sodium and water reabsorption in the renal medulla [31, 32]. The relevance of the endothelin system for the kidney under basal conditions is not well established. Usually no or only little effects on renal blood flow and sodium excretion have been observed under basal conditions during intrarenal endothelin blockade in animals [33–35], while one study demonstrated an increase in renal blood flow and sodium excretion [36].

Pharmacological antagonism of the endothelin system

A major advance in our understanding of the renal endothelin system was made by the development of pharmacological antagonists of ET-1. Currently drug development in this area focuses on reducing endogenous ET-1 production by inhibition of endothelin converting enzyme (ECE), and antagonizing binding of ET-1 to its receptor.

ECE is a pH neutral metalloprotease that is inhibited by phosphoramidon but is insensitive to related metalloprotease inhibitors such as thiorpan [37]. In agreement, phosphoramidon inihibits release of ET-1 from endothelial cells and antagonizes the pharmacological effects of exogenously administered ET-1 in a variety of animal protocols as well as in the human forearm [38, 39]. However, phosphoramidon is also a potent inhibitor of NEP 24.11, an enzyme which is widely distributed in the body and which is involved in the clearance of ET-1 as well as in the metabolism of bradykinins and ANP [23]. These actions challenge the use of phosphoramidon as a selective ECE-inhibitor. Therefore, several pharmaceutical companies are developing more selective ECE-inhibitors based on phosphoramidon or big ET-1 analogues [40–42].

The development of ET receptor blockers has been established on development of peptide analogues. The discovery of BQ123, a cyclic pentapeptide which selectively inhibits the ETa receptor,

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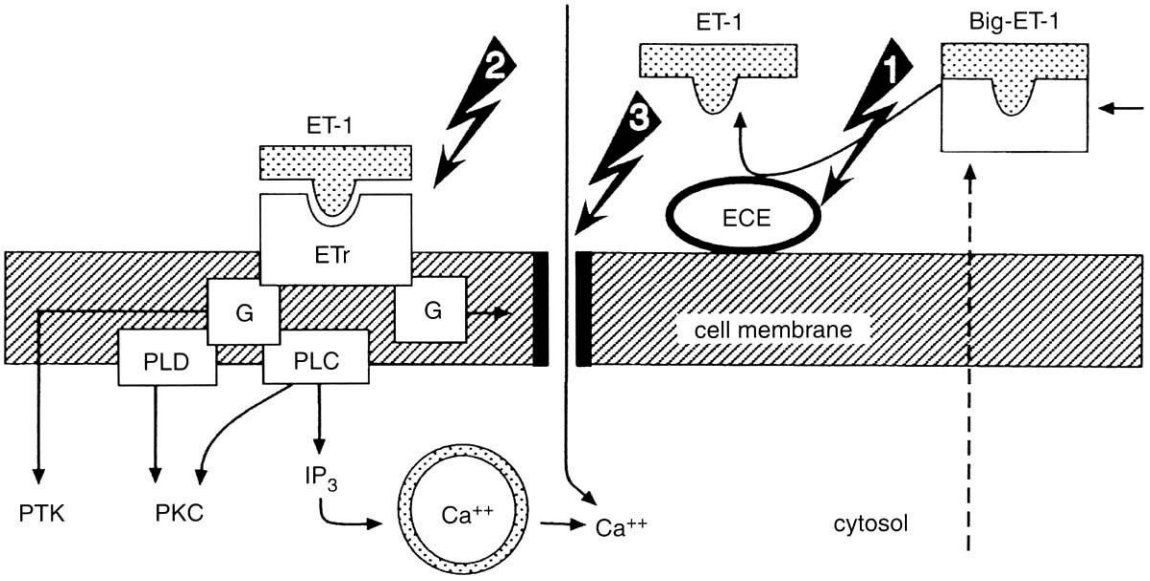

Fig. 1. *Schematic representation of signal transduction and targets for antagonism of endothelin.* Big ET-1, secreted by the target cell or derived from the circulation, is converted by endothelin converting enzyme (ECE) to activate ET-1. Arrow 1 indicates the site of action of ECE inhibitors. ET-1 subsequently binds to the receptor (ETr) and activates phospholipase C via a pertussis-toxin insensitive G-protein (G), resulting in the formation of inositol triphosphate (IP3). This IP3 formation will stimulate intracellular calcium mobilization, which activates L-type calcium channels, thus contributing to sustained increments in intracellular calcium. ETr activation perhaps may also directly open these channels. The receptor can be blocked by receptor antagonists (arrow 2), and the L-type calcium channels by calcium channel blockers (arrow 3). It has been postulated that these signal transduction pathways are mainly involved in the contractile actions of ET-1. ET receptor activation will also stimulate protein kinase C (PKC), and several protein tyrosine kinases (PTK). These cytosolic effectors in turn alter gene expression, and have been implicated in the mitogenic actions of ET-1, while PKC may perhaps also contribute to vasoconstriction by phosphorylation of myosin light chains. Finally, ET receptor activation can stimulate arachidonic metabolism via activation of phospholipase A_2 (not shown).

Table 1. Orally available ET-receptor antagonists in clinical drug development

Compound	Selectivity	Company
Bosentan	mixed ETA/ETB	Hoffman-LaRoche
SB 209670	mixed ETA/ETB	SmithKline Beecham
BMS 182874	ETA	Bristol-Myers Squibb
LU 135252	ETA	Knoll
A-127722	ETA	Abbott

signified a major progression in this field [43]. Later hexapeptides were developed based upon the C-terminal portion of the endothelins, which could block both the ETa and the ETb receptor. A major problem with these peptide-analogues is that they are not effective via the oral route. Moreover, binding of peptide analogues to ET receptors is less stable than the binding of native endothelins to these receptors [44], which may render these antagonists less effective. This explains the high dosages required in experimental studies. These characteristics and the high costs of the peptide analogues make these receptor antagonists less suitable for use in humans. Only recently have non-peptide ET receptor antagonists been introduced, some of which are also orally active (Table 1). Drug development in this area is rapidly evolving and by the time this review is being published new compounds probably will have been disclosed.

Several other ways to interfere with the renal endothelin system are under investigation as well. For example, there is increasing evidence that ET-1 may also interact with ATP-sensitive potassium channels [45–47]. This could constitute a target for future pharmacological antagonism of ET-1. Therapeutic strategies based on functional antagonism of ET-1 induced constrictor or growth promoting effects may emerge as well. For example, the natriuretic peptides such as ANP and BNP, as well as inhibitors of their degradation may directly or indirectly oppose ET-1 under certain conditions [48, 49]. Functional antagonism of ET-1 by nitrovasodilators or stimulation of endogenous nitric oxide has also been reported, although this mechanism appears to be less efficacious in the human kidney [30, 50].

Endothelin as a pathophysiological factor in renal disease

Elevated plasma levels of ET-1, secondary to decreased renal clearance of the peptide, have been reported in many renal diseases [51–53]. In addition, renal production of ET-1 may be enhanced in renal diseases, even when circulating ET-1 is not elevated [54–57]. Both circulating levels of ET-1 as well as locally produced ET-1 can cause long-lasting increments in renal vascular resistance via preglomerular and postglomerular constriction [58–61]. In addition, ET-1 can cause mesangial contraction and thus reduce the ultrafiltration coefficient [28]. These vasoconstrictive effects of ET-1 were confirmed *in vivo* in humans, where infusion of ET-1, attaining pathophysiological increments in plasma ET-1 levels, caused profound and prolonged vasoconstriction [29, 62]. ET-1 may thus play a role in the development of acute renal failure, which most commonly arises due to renal vasoconstriction and subsequent ischemic necrosis of renal tubules [63]. In agreement with this hypothesis, plasma levels of ET-1 and/or renal expression of ET-1 have been reported to be elevated in acute

ischemic renal failure [51], in septicemia [64] and during administration of cyclosporine and radiocontrast agents [65–67]. Moreover, acute inhibition of the actions of ET-1 by administration of antibodies against ET-1 or endothelin-receptor blockers reduce renal vasoconstriction and glomerular dysfunction in experimental renal ischemia [68–72], and can decrease mortality among treated animals even when administered 24 hours after the ischemia [73]. Similarly, endothelin-receptor blockers also could restore cyclosporine-induced changes in renal hemodynamics and blood pressure [71, 72, 74].

Endothelin may also contribute to progressive structural renal damage in chronic kidney disease. In rats with partial renal mass (by ablation), urinary excretion of endothelin was markedly enhanced [55–57] and it increased in time parallel to the development of glomerulosclerosis. Similarly, patients with chronic renal disease demonstrate significantly elevated urinary ET-1 excretion [75]. Development of glomerulosclerosis in the renal ablation model, as well as in models such as puromycin-nephrosis [76], and streptozotocin-induced diabetes [77] was also accompanied by increased ET-1 gene expression in the kidney, suggesting that ET-1 may also play a role in the progression of chronic renal failure. Indeed, in analogy to other vasoactive peptides, such as angiotensin II, ET-1 has been demonstrated to stimulate mesangial cell growth *in vitro* [24–26] as well as *in vivo* [78] and to increase the expression of extracellular matrix proteins such as collagen and laminin [79, 80]. Both compensatory renal growth and matrix expansion are characteristic of progressive renal disease. In addition, several other factors that have been implicated in the pathogenesis of progressive renal disease, such as angiotensin II, transforming growth factor-β, insulin-like growth factor and basic fibroblast growth factor, stimulate the production of ET-1 [13, 14]. A role for endothelin in the pathogenesis of chronic progressive renal disease is reinforced by observations showing that endothelin-receptor blockade could reduce the development of proteinuria and glomerular lesions in the renal ablation model [81, 82] as well as in experimental immune complex nephritis [83]. These results still require further verification, but clearly indicate an exciting therapeutic application of the endothelin concept.

Renal disease is also frequently complicated by hypertension, which has been associated with accelerated development of further renal injury. Because of its vasoconstrictor action and its effects on vascular hypertrophy, ET-1 has also been implicated in the pathogenesis of hypertension and/or the maintenance of hypertension. Persistent hypertension may occur following infusion of ET-1 *in vivo* and as a result of endothelin secreting tumors [84, 85]. In addition, the preliminary data on systemic ET-receptor blockade in humans show vasodilation and reduction of blood pressure, suggesting that ET-1 plays a role in the maintenance of vascular tone [86]. In some animal models of hypertension, such as the sodium-deplete squirrel and the DOCA salt hypertensive rat, ET receptor blockade caused marked reductions in blood pressure [87, 88]. In the latter model this was also associated with regression of vascular hypertrophy. On the other hand, the effects of ET antagonism in other experimental models of hypertension, notably the SHR, are less clear [reviewed in 89]. The possible role ET-1 as a hypertensive factor in renal disease is not yet well established. However, administration of endothelin receptor blockers in experimental models of renal disease, such as the renal ablation model and chronic administration of cyclosporine [74, 81], causes large reductions in arterial blood pressure, suggesting that hypertension in some models of renal disease may be largely ET-dependent.

Endothelin receptor blockade in human renal disease

The renal endothelin system offers an attractive pathophysiological paradigm by implicating a new pathophysiological factor in the development of both acute and chronic progressive renal disease. The final answer to whether or not ET receptor blockers will be beneficial in human renal disease can be obtained only by short-term and long-term studies in patients. However, before reaching that stage, several issues relevant for our general expectations concerning the clinical applicability of the endothelin concept in renal medicine should be considered. First is the question of whether ET-1 acts only as a renal pathogen. Second, attention to species differences with regard to the renal endothelin system between experimental animals and humans is crucial because of the consequences that such differences may have for disease and for therapeutic approach. Third, we should determine if ET-1 has a central role among the many known (and unknown) pathogenetic factors, or whether it plays no more than a sideline role, since this will determine its pathogenetic relevance. Finally, it must be kept in mind that functional effects of ET receptor blockers should also result in better kidney survival.

It is important to consider whether an activated intrarenal endothelin system has only deleterious actions. In normal kidneys ET-1 immunoreactivity is largely confined to the vascular and glomerual endothelium, whereas there is no ET-1 present in the tubules [12]. However, tubular ET-1 production can be induced *in vitro* by ischemia. Ong et al showed in cultured human proximal tubule cells that such ET-1 production is amplified through activation of tubular ETb receptors [90]. Activation of these tubular ETb receptors also stimulates mitogenesis, which may contribute to tubular regeneration after ischemia. This would suggest a protective role for ET-1 and ETb-receptor activation in some forms of renal disease. The relevance of this hypothesis is supported by *in vivo* observations in the dogs, where moderate hypoxia also resulted in increased ET-1 immunostaining in proximal and distal tubules. This concurred with increased urinary ET-1 excretion and fractional sodium excretion [91], suggesting that ET-1 may also play a role in sodium and water homeostasis under these conditions. In agreement, ET-1 could inhibit Na-K-ATPase in proximal tubules and decrease O_2 consumption in inner medullary collecting duct cells [31]. Clavell et al showed that these effects of ET-1 in the dog are also mediated through activation of the ETb receptor: intrarenal infusion of ET-1 in dogs caused vasoconstriction and sodium retention, whereas ET-1 infusion in the presence of the ETa receptor blocker BQ 123, thus allowing selective stimulation of the ETb receptor, had no effects on renal hemodynamics and resulted in diuresis and natriuresis [92]. Such studies suggest that under certain conditions, such as ischemia, ET-1 may also serve as a compensatory system to maintain sodium and water excretion, and favors tubular regeneration. The relevance of such potential actions of the ET system is underscored by observations that the renal medullary ETb and not the vascular ETa receptor is up-regulated in experimental models of glomerulonephritis, hypertension, cyclosporine nephropathy and liver cirrhosis [93–96]. Indeed, combined ETa/ETb receptor blockade in experimental liver cirrhosis resulted in

impaired water excretion, which is an unwanted side effect in the clinical setting of this condition [96].

Regarding the function of the ET receptor subtypes, important species differences probably exist. In rats the ETb receptor appeared to be crucial in mediating the renal vasoconstrictive effects of ET-1 [97–99], whereas in the dog ET-mediated vasoconstriction was ETa receptor dependent [92]. Such differences may explain why sometimes the combined ETa/ETb receptor blockade is more effective in rat models of renal disease than selective ETa receptor blockade. In humans ETb receptors may mediate vasoconstriction in some vascular beds [100, 101]. However, the human kidney probably is not subject to important ETb receptor mediated vasoconstriction, since infusion of the selective ETb receptor agonist ET-3, in an equimolar amount to dosages of ET-1 that cause profound renal vasoconstriction, has no effect on renal perfusion [102]. In these normal subjects ET-3 infusion had no effects on sodium and water excretion. However, this does not exclude such actions in disease states. Taken together, these data cannot identify an important constrictor action of ETb receptor activation in the normal human kidney, while there are experimental indications that under certain conditions ETb-receptor blockade could be harmful. This underscores the need for further information on the role of the ETb-receptor in human renal disease when applying ET-receptor blockers clinically. This is also of relevance for conditions such as heart failure, which has currently been indentified as a major target for ET-receptor antagonists development [103].

The specificity of the endothelin as a renal pathogen also remains to be established. Renal disease is a multifactorial process. For example, development of acute renal failure involves important mechanisms such as ATP depletion, cellular calcium overload and reperfusion injury [104, 105]. It is feasible that the renal vasodilator actions of ET-receptor blockade can ameliorate part of the ischemic component of acute renal failure. However, calcium channel blockers can also reverse and largely prevent ET-1-induced renal vasoconstriction in humans [29, 106, 107]. In addition these drugs may also have effects on the other mechanisms involved in the pathogenesis of acute renal failure [108]. When considering the pathophysiological actions of ET-1 as a cytokine, one has to realize that multiple interactions also exist between endothelin and other renal growth factors such as PDGF, vasopressin, fibroblast growth factor and epidermal growth factor [78, 109–112]. The hierarchical role of endothelin within this complex is still unknown. This is of great importance to evaluate its pathogenetic role. If endothelin would be a downstream effector molecule of other vasoactive peptides or cytokines, then inhibition of endothelin may also ameliorate the pathogenic actions of such factors. In favor of this possibility, the hypertrophic effects of angiotensin II in cardiac myocytes could be modulated by endothelin-receptor blockade [113]. One study recently reported that the endothelin blockade could reduce the proliferative actions of PDGF on mesangial cells, indeed suggesting a more central role for ET-1 in the development of renal fibrosis [110]. However, currently this central role for ET-1 in renal fibrosis *in vivo* remains to be established. Such information may not only help to define the pathogenetic potential of endothelin, but may also help to indentify conditions apart from primarily elevated plasma endothelin levels that may benefit from endothelin receptor blockade (such as, such as activation of the renin-angiotensin II system).

Finally, most of the preclinical studies, and also the few human data that are available, focus on acute changes in renal hemodynamics in acute experimental conditions, as artificial end points. Although such studies may be of relevance for the pathophysiology of acute renal failure, they do not reflect the pathophysiological mechanisms involved in most renal diseases, where renal outcome is more likely to be determined by structural damage. Although renal hemodynamic dysfunction and renal fibrosis are linked in many different ways, and changes in renal perfusion often precede or contribute to development of structural damage, there may also be dissociations between hemodynamic changes and structural damage. This was recently shown in chronic cyclosporine nephrotoxicity, an outstanding example of renal injury in which ET-1 has been incriminated in the pathogenesis [3]. During chronic administration of cyclosporine, both ETa and combined ETa/ETb blockade could still ameliorate glomerular dysfunction [114, 115]. Nevertheless, structural damage such as tubular dilation and vacuolization and arteriolopathy did not change, while tubulointerstitial fibrosis even worsened during the endothelin blockade [114, 115]. Such data underscore the necessity of evaluating the potential of endothelin receptor blockers to prevent structural renal damage and improve (renal) survival in models of chronic progressive renal disease.

Conclusion

Endothelin is probably a two-edged sword to the kidney. On the one hand it may contribute to renal injury through its actions as a vasoconstrictor and cytokine. On the other hand it may also act as a counterregulatory mechanism serving the maintenance of renal tubular function. Clearly, more information is needed on the relative contributions of these dual actions of ET-1 in renal disease and the ET receptor subtypes involved before applying ET receptor blockers in human renal disease. In view of the species differences with regards to the renal endothelin system, further research on this issue should be focused on the human kidney. The experimental studies thus far have also made clear that the putative effects of the ET receptor blockade on structural renal injury rather than the hemodynamic actions will determine the clinical value of these drugs in renal medicine. Finally, it may be relevant to focus future studies in this field on the potential additional benefits of ET receptor antagonists to standard drugs such as ACE-inhibitors and calcium channel blockers.

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