Biology of endothelin receptors in the collecting duct

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The collecting duct endothelin (ET) system, involving ET-1 and its two receptors, is involved in the physiologic regulation of renal sodium (Na), water, and acid excretion. Based on *in vitro* studies and experiments using genetically engineered rodents, the physiology of this system in the collecting duct is being elucidated. Activation of endothelin B (ETB) receptors on principal cells causes inhibition of Na transport through signaling pathways involving src kinase, MAPK1/2, nitric oxide, and possibly prostaglandin E2 (PGE2). Principal-cell ETB receptors also cause inhibition of water transport through protein kinase C–mediated inhibition of AVP-dependent cAMP accumulation. ETB receptors expressed on intercalated cells augment acid secretion, possibly through nitric oxide–dependent mechanisms. The role of endothelin A (ETA) receptors in the collecting duct remains unclear; however, recent evidence suggests that these receptors can exert natriuretic and diuretic effects. Further complexity is lent to this system by studies indicating that ETA and ETB receptors can homo- and hetero-dimerize, with possible functional consequences. This brief review will describe our current state of knowledge about this complex regulatory system in the collecting duct, and will identify clinically relevant issues that need addressing.

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The collecting duct (CD), by virtue of being the final nephron segment controlling urinary electrolyte and water content, is one of the major renal sites targeted by circulating hormones. Although these factors maintain a vital link between systemic conditions and CD responses, the CD also contains intrinsic systems for maintaining electrolyte and water homeostasis. Of these local mechanisms, CD-derived endothelin-1 (ET-1) and its cognate receptors have emerged as being of particular importance in the control of renal sodium (Na), water, and acid excretion. This review will examine the role of the CD ET system in the modulation of renal function, emphasizing new developments in our understanding of the biology of CD ET receptors.

OVERVIEW OF ET BIOLOGY

The ET family consists of three 21-amino acid peptides (ET-1, ET-2, and ET-3), which are structurally very similar to the snake venom sarafotoxins.[1] ET-1 mRNA encodes a prepropeptide that is cleaved by furin-like proteases to Big ET-1 which is, in turn, proteolyzed by ET-converting enzymes to yield the mature peptide. The kidney contains at least two ET-degrading enzymes, including a neutral endopeptidase. Three ET G-protein-coupled receptors have been described; however, only two have been identified in mammals: the ETA (endothelin A, ETRA) and ETB (endothelin B, ETRB) receptors. ETRA binds ET-1 $\geqslant$ ET-2 $\gg$ ET-3, whereas ETRB binds all ETs with equal affinity. Alternative RNA splicing of both ETRA[2] and ETRB[3] can occur, with alterations in receptor functional characteristics. However, alternative splicing of renal ET receptors, in terms of localization or biological consequences, has not been described.

Antagonists and agonists with apparent high specificity for ETRA or ETRB have been used to help define ET receptor isoform function. Despite the large numbers of studies with these pharmacological agents, unequivocal identification of individual ET receptor isoform action remains elusive. Part of this difficulty may relate to ET receptor dimerization.[4] Fluorescent resonance energy transfer analysis of ET receptors expressed in a heterologous system showed the presence of ETRA and ETRB homodimers.[5] More importantly, this group observed evidence for ETRA–ETRB heterodimerization based on ligand-dependent differences in receptor internalization.[6] Several other studies, using ligand-binding assays or functional readouts of receptor activation, have also suggested that ETRA and ETRB may interact cooperatively.

To further complicate the picture, ETRB may heterodimerize with receptors other than ETRA, including the dopamine D3 and the angiotensin AT1 receptors.[7,8] Whether such ET receptor heterodimerization occurs in the CD is unknown; however, as will be seen from the ensuing discussion, such heterodimerization may indeed occur in the CD and may explain, at least in part, some puzzling results from *in vivo* studies.

CD ET SYNTHESIS AND RECEPTOR EXPRESSION

The CD (particularly the inner medullary CD (IMCD)) produces far more ET-1 than any other cell type in the kidney and possibly in the entire body.[9] CD ET-1 production is increased during natriuretic or diuretic conditions; the mechanisms by which this occur are uncertain, but may include interstitial fluid osmolality, tubule fluid flow rate, and other factors.[9,10]

The CD is also the major nephron site of ET receptor expression. Numerous studies show that the IMCD in experimental animals and humans express ETRB in relatively large amounts.[9,11] Cortical CD (CCD) ETRB expression is also present, as determined by PCR of mRNA, immunostaining, and examination of the effect of ETRB blockade on ET-1 actions in the isolated CCD.[12–19] In contrast, CD ETRA expression has been less clear. Autoradiography of whole kidneys or PCR of nephron segments detected no ETRA in rat or in human CD.[16,20,21] In contrast, immunohistochemical, binding, and PCR studies detected ETRA in the CD from rats or dogs.[22–24] More recent studies, using immunostaining of the rat kidney, observed CD ETRA staining.[25] In addition, ETRA immunoreactivity was detected on the basal infoldings and was scattered within the cytoplasm of the rat CD (particularly in the IMCD).[26] As will be described below, there is also functional evidence for the existence of CD ETRA. Taken together, the above studies suggest that the CD does express ETRA, although in substantially smaller amounts than ETRB.

ET RECEPTORS AND CD Na TRANSPORT AND BP

ET-1 can affect renal Na excretion and blood pressure (BP) through the regulation of a variety of mechanisms, including renal blood flow, glomerular filtration rate, and sodium transport processes throughout the nephron. The CD ET system has proven to be a particularly important physiological regulator of renal Na excretion and systemic BP. Several *in vitro* studies suggested that ET-1 inhibits CD Na transport. ET-1 decreases Na reabsorption in the isolated CCD,[27] reduces Na uptake by distal nephron A6 cells,[28] and inhibits Na/K ATPase activity in IMCD cells.[29] Recent studies, using isolated split-open rat CCD, found that ET-1 decreases epithelial Na channel (ENaC) open probability.[12] Direct evidence that CD-derived ET-1 regulates Na excretion was provided by studies using CD principal cell-specific knockout (KO) of the *ET-1* gene.[30] Mice lacking ET-1 exclusively in the CD were hypertensive ($\sim$18 mm Hg systolic BP greater than controls) and, when exposed to high Na intake, had exacerbated hypertension ($\sim$35 mm Hg systolic BP greater than controls) associated with a reduced ability to excrete the Na load. Amiloride reduced the hypertension in CD ET-1 KO mice, suggesting that the CD was not only the source of ET-1 but may also have been involved in mediating the natriuretic effects of ET-1.

In vitro studies suggested that the natriuretic effects of ET-1 on the CD are largely mediated through the activation of ETRB. In this study, ETRB stimulation inhibited, whereas ETRA activation increased Na uptake by A6 cells.[28] Blockade of ETRB, but not of ETRA, prevented ET-1 inhibition of ENaC open probability in the rat CCD.[12] Intra-medullary infusion of an ETRB antagonist reduced urine flow and Na excretion, an effect that could have been mediated by the blockade of CD ETRB and/or by reducing medullary blood flow.[31,32] In whole animal studies, rats deficient in ETRB, which had been rescued from early mortality by a dopamine β-hydroxylase promoter ETRB transgene, had salt-sensitive hypertension.[33] The hypertension was partially corrected by amiloride, suggesting that CD ETRB inhibition of ENaC is involved in maintaining normal BP. More direct evidence for a role of CD ETRB in modulating BP and Na excretion was obtained using mice with CD principal cell-specific KO of ETRB.[11] These mice had elevated BP; high Na intake further increased BP and was associated with decreased elimination of the Na load. However, the degree of hypertension and Na retention was approximately one-half of that seen with CD ET-1 KO mice on a normal or high Na diet, suggesting that CD ETRB does not mediate all of the anti-hypertensive and natriuretic effects of CD-derived ET-1.

In vitro studies had found no evidence for CD ETRA modulation of CD Na transport; however, to examine this question *in vivo*, mice with CD principal cell-specific KO of ETRA were generated.[34] As expected, CD ETRA KO had no effect on BP or urinary Na excretion, either during normal or high Na intake. Taken together, the CD ET-1 KO, CD ETRA KO, and ETRB KO findings suggested that CD-derived ET-1 exerted natriuretic and hypotensive effects in part through the autocrine activation of CD ETB receptors and in part through the paracrine interaction with cells other than CD principal cells. To confirm this conclusion, mice were developed with combined disruption of ETRA and ETRB in CD principal cells.[35] Contrary to expectations, the magnitude of hypertension and Na retention in CD ETRA/B KO mice was significantly greater than that in CD ETRB KO animals. Furthermore, the degree of Na retention and hypertension in the double ET receptor KO mice was almost identical to that observed in CD ET-1 KO mice. How can these findings be reconciled? Although the reason(s) remain speculative, one possibility relates to ET receptor dimerization. As ETRBs are the most abundant isoform in the CD, then ETRB homodimers and ETRA/B heterodimers may predominate. In the CD ETRB KO, ETRA homodimers, which may not normally exist in sufficient amounts, could potentially exert a natriuretic effect. Although *in vitro* studies do not support such a role for ETRA, very recent studies

suggest that ETRA may, in fact, be natriuretic. Infusion of ET-1 into the renal medulla of ETRB-deficient rats surprisingly caused a natriuresis that was blocked by the administration of an ETRA antagonist.[36] This effect was observed only in female rats; however, ET-1 infusion reduced medullary blood flow (thereby exerting a counteracting anti-natriuretic effect) only in male animals. Clearly, more studies are needed to clarify the role of CD ETRA, although demonstration of ET receptor dimerization *in vivo*, and its functional consequences, will be challenging. It should be noted that clarification of the role of CD ETRA in regulating natriuresis may have immediate clinical relevance. Administration of ETRA antagonists to humans is associated with a high incidence of hemodilution and edema formation, strongly suggestive of renal fluid retention.[37] For the renal community, this issue is of particular importance in that ETRA antagonism with avosentan caused a substantial (~50% vs controls) reduction in urinary albumin excretion in a phase II trial in patients with diabetic nephropathy.[38] A recent phase III trial intended to examine the effect of avosentan on the doubling of serum creatinine, ESRD or death in these patients was discontinued due to problems associated with fluid retention.[39]

The mechanisms by which CD ET receptors reduce Na transport have been partially resolved (Figure 1). ET-1 rapidly decreases rat CCD ENaC open channel probability (within 5 min) through ETRB and this effect is dependent on src kinases and MAPK1/2 (mitogen-activated protein kinase 1/2) signaling, while being independent of phospholipase C or protein kinase C.[12] Nitric oxide (NO) also is involved in the natriuretic and hypotensive effects of ET-1, which, through ETRB activation, stimulates NO synthase (NOS)1-dependent NO production by cultured rat

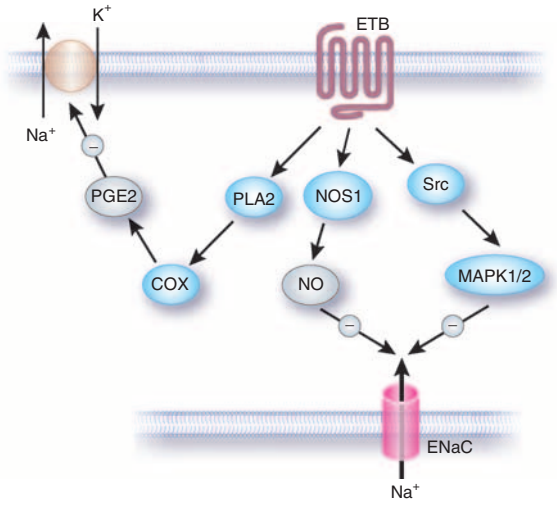

Figure 1 | Schematic of ET receptor B (ETRB) regulation of the collecting duct Na transport. COX, cyclooxygenase; ENaC, epithelial Na channel; MAPK, mitogen-activated protein kinase; NO, nitric oxide; NOS, nitric oxide synthase; PGE2, prostaglandin E2; PLA, phospholipase A; Src, src tyrosine kinase.

IMCD cells.[40] ET-1 increased NOS1 protein expression in the IMCD3 cell line; however, interestingly this was ETRA dependent.[41] Infusion of the ETRB agonist, sarafotoxin S6c, into the rat renal medulla caused a diuresis and natriuresis that was suppressed by the inhibition of NOS1 or protein kinase G.[42] Notably, NO blockade increased the BP in control and in CD ET-1 KO mice to the point at which there was no BP difference between the two groups, whereas urinary nitrate/nitrite excretion and activities of NOS1 and NOS3 were substantially lower in CD ET-1 KO animals.[43] In the same study, incremental increases in renal perfusion pressure were associated with reduced Na and NO metabolite excretion in CD ET-1 KO mice. Finally, a role for prostaglandin E2 (PGE2) in mediating ET-1 natriuresis in the CD was suggested by studies in which COX (cyclooxygenase) blockade prevented ET-1 inhibition of Na/K ATPase activity in the isolated IMCD.[29] ET-1 increases IMCD PGE2 production, and this is carried out through ETRB activation.[29,44] In contrast, COX inhibition in CD ET-1 KO mice did not affect Na retention or BP, although urinary and IMCD PGE2 levels were unexpectedly increased.[45] This increase in PGE2 seems to be a compensatory response for alterations in water metabolism, so it is impossible to rule out a role for CD PGE2 in the ET-1 natriuretic response. In summary, CD ETRB activation with resultant increases in the activity of src kinase, MAPK1/2, NOS1, and possibly COX, inhibits CD Na transport. CD ETRA activation may increase NOS1; however, the biological consequences of this remain to be determined.

ET RECEPTORS AND CD WATER TRANSPORT

There is abundant evidence that the CD ET system modulates renal water transport. Initial *in vitro* studies noted that ET-1 inhibited vasopressin (arginine vasopressin, AVP)-stimulated osmotic water permeability in the CCD and IMCD.[27,46] The physiological role of CD-derived ET-1 in regulating renal water handling was shown using CD ET-1 KO mice.[47] CD ET-1 KO animals had reduced plasma AVP levels, impaired ability to excrete an acute water load, and increased urine concentration in response to exogenous AVP.

The ET receptor subtype in the CD responsible for the diuretic effects of ET-1 has not been ascertained fully, but is likely to be, at least in large part, ETRB. ET-1 blockade of AVP-stimulated osmotic water permeability in acutely isolated CD is ETRB dependent.[27] ET-1 has also been shown to inhibit AVP-stimulated cyclic adenosine monophosphate (cAMP) accumulation in the CD; this effect was due to ETRB activation.[18,44] CD ETRA KO mice have decreased responsiveness to AVP as evidenced by elevated plasma AVP levels and an enhanced ability to excrete a water load; this suggests that CD ETRA may exert an anti-diuretic effect.[34] This conclusion is further supported by the finding that the IMCD isolated from CD ET-1 KO mice had elevated AVP-stimulated cAMP accumulation, whereas IMCD suspensions from CD ETRA KO mice had reduced AVP-stimulated cAMP accumulation. Similar studies on water handling and cAMP

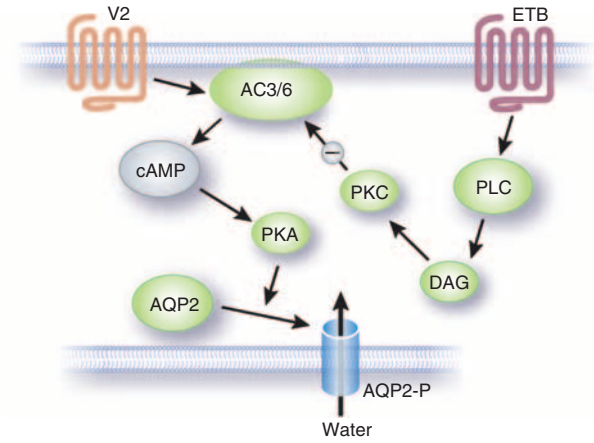

Figure 2 | Schematic of ET receptor B regulation of the collecting duct water transport. AC3/6, adenylyl cyclase 3 and 6 isoforms; AQP2, aquaporin-2; AQP2-P, phosphorylated AQP2; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; V2, vasopressin receptor.

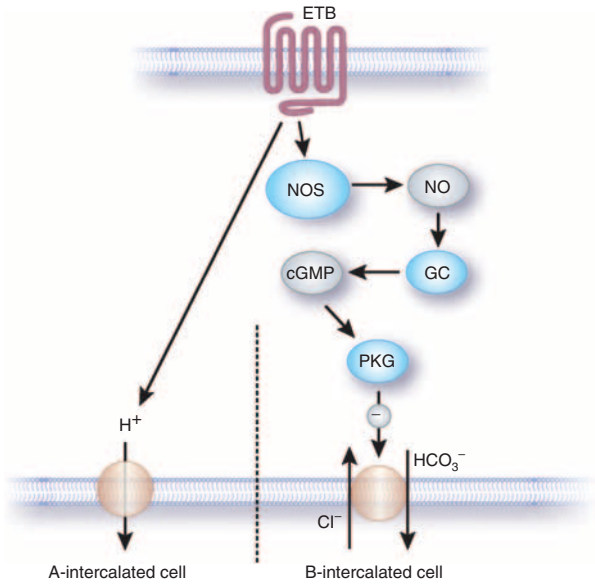

Figure 3 | Schematic of ET receptor B regulation of the collecting duct proton and bicarbonate transport. cGMP, cyclic guanosine monophosphate; GC, guanylyl cyclase; NO, nitric oxide; NOS, nitric oxide synthase; PKG, cGMP-dependent protein kinase.

production are underway in CD ETRB and in combined CD ETRA/B KO mice.

The mechanisms by which ET-1 inhibits CD water transport are partially understood (Figure 2). ETRB-mediated inhibition of AVP-stimulated cAMP content in the IMCD is dependent on phospholipase C, diacylglycerol, and protein kinase C, and likely involves interaction with adenylyl cyclase isoforms 3 and 6.[18,44] ET-1 acts through an inhibitory G protein as pertussis toxin blocks the ET-1 effect on AVP-stimulated cAMP accumulation in the IMCD.[44] As ET-1 stimulates CD NO production and as NO can inhibit AVP-stimulated water permeability and cAMP accumulation in the rat CCD,[48] it would seem likely that NO at least partially mediates the ET-1 diuretic effect. However, NOS blockade had no effect on ET-1 inhibition of AVP-stimulated cAMP content in IMCD suspensions.[40] Notably, no effect of NO donors on AVP-stimulated cAMP levels was detected in rat IMCD cells. Why different results were obtained in these studies is unknown; it remains, therefore, an open question as to whether NO mediates, at least in part, the diuretic effects of ET-1 in the CD.

ET RECEPTORS AND CD ACID–BASE SECRETION

ET-1 may be involved in enhanced urinary acidification in response to a systemic acid load.[49] Metabolic acidosis stimulates renal ET-1 production;[49] the specific cell types involved are not known fully. Preliminary studies in our laboratory found that mice with intercalated cell-specific KO of ET-1 developed metabolic acidosis, suggesting that ET-1 derived from this cell type may be involved in maintaining normal acid secretion (unpublished results). In addition, ET receptor antagonism reduced luminal acidification in the isolated CCD (that is, endogenous ET-1 effects were blocked).[19] This latter study found that ETRB blockade prevented the adaptive increase in H^+ secretion by A-intercalated cells and the decrease in HCO_3^- secretion in B-intercalated cells in response to an acute (3 h) decrease in luminal and bath pH to 6.8. Furthermore, the adaptive decrease in HCO_3^- secretion, but not that in H^+ secretion, was prevented by NOS, guanylyl cyclase, or protein kinase G blockade (Figure 3). Although direct identification of the relevant signaling pathways is needed, taken together, the above studies increase the possibility that CD-derived ET-1 acts, possibly through ETRB, on both A- and B-intercalated cells, and NO in B-intercalated cells, to augment CD luminal acidification.

ALTERATIONS IN CD ET RECEPTORS IN DISEASE STATES

Relatively little is known about changes in CD ET receptors in disease states. CD ETRB expression was found to be increased in rats with compensated congestive heart failure, whereas CD ETRB expression was substantially downregulated in rats with decompensated heart failure.[50] Although the functional significance of such changes remains speculative, decreased CD ETRB might contribute to Na retention in decompensated heart failure. CD ETRA and ETRB expression were not changed in patients with acute renal transplant rejection (although distal tubule ETRA labeling was decreased).[51] Clearly, more studies are needed on the role of alterations in CD ET receptors in disease states.

SUMMARY

The ET system is involved in modulating Na and water reabsorption by CD principal cells, and possibly H^+ and HCO_3^- secretion by CD intercalated cells. CD ETRB appears to mediate ET-1 inhibition of ENaC, AVP-stimulated water transport, and acid-induced urinary acidification. Findings in experimental animals, and in humans treated with ETRA

antagonists, suggest that CD ETRA may exert natriuretic and diuretic effects.

Despite our advances in understanding the CD ET system, key questions remain to be answered. If the CD ET system is fundamentally important in fine-tuning renal Na, water and acid excretion, how can ET-1 production and CD ET receptor activation be tightly controlled and compartmentalized so that transport of these substances is individually regulated? Does ET receptor homodimerization and/or heterodimerization occur, how is it regulated, and does such a dimer configuration exert functional effects? As the clinical use of ETRAs grows, associated with side effects that may involve the kidney (fluid retention), the answers to these questions become increasingly pressing.

DISCLOSURE

The author declared no competing interests.

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