Regulation of Sodium Transport in the Proximal Tubule by Endothelin

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Abstract

Human essential hypertension and rodent genetic hypertension are associated with increased sodium transport in the renal proximal tubule and medullary thick ascending limb of Henle. The proximal tubule, which secretes endothelin (ET), expresses the ET_B receptor. Low (nM) concentrations of ET, via the ET_B receptor, inhibit sodium and water transport and ATP-driven drug secretion in the proximal tubule. In contrast, very low (pM) and high nM concentrations of ET increase renal proximal sodium transport, but the receptor involved remains to be determined. The natriuretic effect of ET_B receptor stimulation is impaired in spontaneously hypertensive rats, due in part to a defective interaction with D₃ dopamine and angiotensin II type 1 receptors. Impaired ET_B receptor function in the renal proximal tubule may be important in the pathogenesis of genetic hypertension.

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The kidney is important in the long-term regulation of blood pressure and is the major organ involved in the regulation of body sodium homeostasis [1–3]. The proximal tubule and medullary thick ascending limb of Henle are pre-eminent in the overall regulation of sodium balance in essential hypertension [4]. Indeed, several studies have shown that human essential hypertension and rodent genetic hypertension are associated with increased sodium transport in the renal proximal tubule and medullary thick ascending limb of Henle [4].

Endothelin (ET) was initially identified as an endothelial cell-derived peptide, with the greatest vasoconstrictor potency of any known endogenous compound [5]. ETs are a family of isopeptides (ET-1, ET-2, and ET-3), with at least

two receptors subtypes (ET_A and ET_B). Renal tissue expresses both ET receptor subtypes [6], and ET is secreted by renal tubules, including the renal proximal tubule [7, 8], where it can regulate sodium transport in an autocrine/paracrine manner [9]. In the renal proximal tubules, ET-1 (the major ET expressed in the renal proximal tubule) inhibits ion transport principally through the ET_B receptor [10], an effect opposite that of the ET_A receptor. This review focuses on the regulation of ET on sodium transport in the renal proximal tubule and its role in the pathogenesis of essential hypertension.

Endothelin and Its Metabolism

In 1985, Hickey et al. [11] described the existence of a trypsin-sensitive endothelium-derived constricting factor in cultured bovine endothelial cells, which they named as ET. Subsequently, more ET family members were found. These members include ET-1, ET-2, ET-3, and ET-4 (analogue of human ET-2 in rat and mouse, also named as a vasoactive intestinal contractor), as well as three isoforms of 31-amino-acid ETs ($ET-1^{1-31}$, $ET-2^{1-31}$, and $ET-3^{1-31}$) [12]. Owing to the similarity of actions between $ET-1^{1-31}$ and ET-1 in vivo, it is possible that some of the effects of $ET-1^{1-31}$ result from their partial bioconversion into ETs [13]. Among these isoforms, ET-1 is the principal isoform and is the most potent and long-lasting constrictor of human vessels known to date [5].

ET-1 is produced in many cell types in the renal and cardiovascular systems, such as endothelial cells, smooth muscle cells, cardiomyocytes, leucocytes, macrophages, and renal tubular and mesangial cells [14, 15]. Bioactive ETs are the product of post-translational processing of the parent preproET peptide. The transcription and translation of *preproET* result in the formation of a 203-amino-acid peptide which is subsequently cleaved by a furin convertase to the 38-amino-acid peptide big ET^{1-38} . Big ET is processed further into ET^{1-31} by different isoforms of ET-converting enzymes (ECEs), a group of proteases that belong to the metalloprotease family [16], including ECE-1a, ECE-1b, ECE-1c, and ECE-1d, derived from a single gene by the action of alternative promoters.

ET synthesis is regulated by many factors. It is enhanced in response to low-shear stress, turbulent blood flow, hypoxia, cytokines, angiotensin II, epinephrine, and low-density lipoproteins [17]. In contrast, high-shear stress, nitric oxide, vasodilating prostaglandins, and natriuretic peptides suppress ET production [18]. ET synthesis is regulated by sodium diet; a high-sodium diet, independent of blood pressure status, increases renal synthesis of ET [19]. Distal nephron segments synthesize ET-1 to a greater extent than the proximal tubule [8].

Synthesized ET-1 is released in two ways. One way is via a constitutive pathway, producing intense constriction of the underlying smooth muscle, which contributes to the maintenance of endogenous vascular tone. The other way is via release from endothelial cell-specific storage granules (Weibel-Palade

bodies) in response to external physiological stimuli, producing further vasoconstriction [20]. Although plasma ET-1 is present in the highest concentration in blood/plasma, compared with ET-2 and ET-3, ET-1 concentrations are still lower in plasma than in endothelial and other cells. It is accepted that ET-1 functions as a locally released, rather than a circulating, hormone.

Endothelin Receptors

Endothelin Receptor Classification

ET receptors are classified as ET_A and ET_B by the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification [21]. Both ET_A and ET_B receptors belong to the 7-transmembrane domain or G protein-coupled rhodopsin-type receptor superfamily. Pharmacologically heterogeneous responses seem to be related to the existence of alternatively spliced variants of ET_A and ET_B receptors. A third receptor, named ET_C, which is specific for ET-3 binding, was cloned from dermal melanophores from the amphibian *Xenopus laevis*, but a mammalian homologue has yet to be identified [21] (see below).

The three ET receptors have different affinities to the three isoforms of ET (ET-1, ET-2, and ET-3). ET receptors can bind all ET isoforms, but the ET_A receptor has a much higher affinity for ET-1, the most abundant ET in human plasma, than for ET-3, while the ET_C receptor has a higher affinity for ET-3 than ET-1. In contrast, the ET_B receptor binds to all three ET isoforms with equal affinity.

Localization of Endothelin Receptors

In all species studied, including humans, ET-binding is greater in the renal inner medulla than in the inner cortex [8, 22, 23].

In the rat, there is faint immunostaining of the ET_A receptor in the proximal convoluted and straight tubules, restricted to the basal side, and intense staining in the distal tubule collecting duct [24, 25]. The ET_A receptor is also expressed in mesangial cells, pericytes of descending vasa recta, and vascular smooth muscle cells of veins and arteries, specifically the interlobar, arcuate, and interlobular arteries, as well as efferent and afferent arterioles [25]. The ET_B receptor is the major ET receptor in the kidney; 70–80% of ET receptors in the kidney are ET_B receptors [6, 8], which in the rat are expressed in the proximal tubule, inner medullary collecting duct, glomerular capillaries, vasa recta endothelial cells, and vascular smooth muscle cells of interlobular, efferent, and afferent arteries [25–28].

In the mouse, the ET_A receptor has been shown to be expressed in some proximal tubules and vessels, but not in glomeruli. In agreement with the rat studies, the ET_B receptor is expressed in the proximal tubule and collecting duct [29].

Faint staining of ET_A and ET_B receptors has also been reported in human glomeruli, and proximal and distal tubules. In agreement with the rat studies, the distal nephron expresses more ET receptors than the proximal nephron [30, 31].

Signal Transduction of Endothelin Receptors in the Renal Proximal Tubules

Both ET_A and ET_B receptors are linked to Gq/11, Gα_s, and Gα_{i/o} [32, 33]. In some cells (e.g. Chinese hamster ovary cells), the ET_A receptor is linked to Gα_s while the ET_B receptor is linked to Gα_i [34]. However, in hepatocytes, the ET_B receptor is also linked to Gα_s [35]. The ET_B, but not the ET_A, receptor also activates Gα₁₃ [36].

In rat renal brush border membranes, the ET-mediated increase in phospholipase C activity and protein kinase C translocation to the brush border membrane are mediated by the ET_B but not the ET_A receptor. In contrast, the ET_C receptor is involved in ET-mediated signaling in basolateral membranes [37]. Both the ET_A and ET_B receptors belong to the class A receptors of the G protein-coupled receptor family because they bind to β-arrestin 1 with a higher affinity than β-arrestin 2, and do not bind to visual arrestin [38, 39].

Effect of Endothelin on Renal Proximal Tubule Transport

In vivo Studies

Low-dose infusion of ET in anesthetized rats has been reported to decrease sodium transport in proximal and distal nephron segments, assessed by lithium clearance, which is associated with an increase in renal blood flow, but not glomerular filtration rate [40]. At a dose that does not alter renal plasma flow, ET also decreases proximal but not distal tubule sodium reabsorption, also assessed by lithium clearance [8, 41, 42]. This shows that ET infusion causes natriuresis without altering glomerular filtration rate or renal blood flow, and suggests a direct inhibitory effect of ET-1 on Na⁺ transport along the nephron. However, ET-mediated natriuresis has not been consistently observed [43, 44]. One group reported that the natriuretic effect of exogenous ET-1 is due solely to an increase in blood pressure since renal decapsulation or maintaining renal perfusion pressure at baseline values (ET agonists often increase arterial pressure) with an aortic clamp prevents ET-1-induced natriuresis [43]. The possible explanation for the inconsistent findings on ET agonist-induced natriuresis may be due, at least partly, to differential activation of ET_A and ET_B receptors. For example, when the ET_A receptor, but not the ET_B receptor, is blocked, a natriuretic effect of ET-1 is detected [45]. One study showed that low doses of ET causes a natriuresis by decreasing renal proximal tubular reabsorption that is not due to ET_B receptors [46].

In contrast to the studies on sodium excretion, there is unanimous agreement that systemically administered ET increases urinary water excretion. Even when given at doses that markedly decreased renal blood flow, the renal arterial infusion of ET-1 increased urine volume and free water clearance [47]. This effect may be mediated by the ET_B receptor because the infusion of ET_B-specific agonists (IRL1620 or sarafotoxin 6c) increases urine flow [10, 46].

The ET_B receptor also works as a clearance receptor. The ET_B receptor in endothelial cells removes ET-1 from the circulation [48, 49]. Blockade of the ET_B receptor increases circulating immunoreactive ETs (ET-1 and ET-3), and mice with genetic ablation of the ET_B receptor in endothelial cells have elevated plasma concentrations of ET-1 [48, 49].

In vitro Studies

ET has diverse effects throughout the nephron. In the rat proximal tubule, ET has a biphasic effect on ion and fluid transport [50]. Low concentrations (pM) increase, whereas a high concentration (low nM) of ET-1 decreases fluid transport through protein kinase C-, cyclooxygenase- and lipoxygenase-dependent mechanisms [42, 50]. However, higher concentrations (high nM) of ET also increase renal proximal tubule transport [7]. In agreement with the data on sodium transport, low nM concentrations of ET, via the ET_B receptor, have also been shown to inhibit the secretion of fluorescent substrates in renal proximal tubules of the killifish [51].

ET-1 inhibits fluid and bicarbonate absorption in the isolated-perfused rat proximal straight tubule [52] and fluid transport in the midproximal convoluted tubule measured by the split-drop micropuncture method [53]. A preliminary report also indicates that ET decreases sodium-phosphate cotransport in rat renal brush border membranes [53, 54]. Garvin and Sanders [52] showed that the ability of ET to inhibit fluid and bicarbonate transport in the rat proximal straight tubule is mediated by a reduction in Na⁺-K⁺ ATPase activity. These effects are probably mediated by the ET_B receptor because stimulation of the ET_B receptor also inhibits Na⁺-K⁺ ATPase activity in immortalized rat S1 renal proximal tubule cells [55], similar to that observed in proximal tubules, with a reduction of about 20–40% [52, 53]. The inhibitory effect of ET, via the ET_B receptor, on Na⁺-K⁺ ATPase activity in human and rat renal proximal tubule cells is mediated by an increase in intracellular calcium via phosphatidyl inositol-3 kinase [55]. However, in suspensions of rabbit proximal tubule cells, ET was not found to reduce oxygen consumption, which is used as an index of Na⁺-K⁺ ATPase activity [56]. It remains to be determined whether or not the differential effect of ET on Na⁺-K⁺ ATPase activity between rats and rabbits is related to species differences.

The effect of ET on NHE3 activity in renal proximal tubule cells is still controversial. Short-term (<1 h) incubation of rat renal cortical slices with ET increases NHE3 activity that is mediated by protein kinase C and inhibition

of cAMP production [57]. Short-term (<1 h) stimulation with ET of opossum kidney cells which express ET_B but not ET_A receptors, also increases NHE3 that is mediated equally by Ca²⁺-dependent and tyrosine kinase-dependent pathways [58, 59]. However, long-term (≥6 h) stimulation of the ET_B receptor in the same opossum kidney cells leads to inhibition of NHE3 activity and expression [60]. One may conclude from these studies that in the renal proximal tubule, ET, via the ET_B receptor, inhibits sodium transport by decreasing Na⁺-K⁺ ATPase activity. In contrast, ET, via the ET_B receptor, acutely stimulates, but chronically inhibits NHE3 activity. However, the end-result of ET_B receptor stimulation should still be a decrease in renal proximal tubule transport because of the inhibition of Na⁺-K⁺ ATPase activity.

Interaction with Other G Protein-Coupled Receptors

Interaction between Endothelin A and Endothelin B Receptors

ET_A and ET_B receptors may exist as constitutive homodimers [61]. They may also exist as constitutive heterodimers [62]; therefore, there may be a 'cross-talk' between these two ET receptors. The ET_B receptor may be essential in regulating the development or expression of the ET_A receptor because ET_A receptor expression is decreased in central or peripheral tissues of ET_B receptor-deficient mice [63]. ET_A and ET_B receptor heterodimers may be responsible for a sustained Ca²⁺ signaling by delaying their internalization [64]. Both the ET_A and ET_B receptors are required for the full diuretic and natriuretic actions of ET associated with the intramedullary hyperosmotic saline infusion [65]. An increased ratio of ET_A and ET_B receptor activity is important in the development and progression of DOCA-salt-induced hypertension and organ damage [66]. Indeed, blockade of ET_B receptors in this model of hypertension increases the severity of vascular and renal proximal tubular damage [66].

Interaction with the D₃ Dopamine Receptor

Dopamine, an endogenous catecholamine, is an important regulator of sodium balance and blood pressure via renal and nonrenal mechanisms, including the regulation of appetite centers in the brain, secretion/release of hormones, and humoral agents, as well as interaction with hormones that regulate renal ion transport. Dopamine receptors are divided into D₁- and D₂-like subtypes based on their interaction with the effector enzyme adenylyl cyclase. Among D₂-like receptor subtypes, the D₃ receptor, along with the D₄ receptor, has the highest affinity for dopamine. Stimulation of the D₃ receptor increases sodium and water excretion in normotensive rats [67, 68]. The natriuresis caused by D₃ receptor stimulation is due, in part, to inhibition of Na⁺-K⁺ ATPase activity via a cooperative interaction with ET_B receptors [28]. The D₃ and ET_B receptors colocalize and physically interact in rat renal proximal tubules and increase each other's expression (fig. 1).

Fig. 1. Interactions among ET_B (ET_B-R), D₃ dopamine (D₃-R), and AT₁ (AT₁-R) receptors in the regulation of sodium transport in the renal proximal tubule. Stimulation of the AT₁-R increases the activity of sodium transporters, sodium exchangers, and the sodium pump (e.g. NaHCO₃ cotransporter, Na⁺H⁺exchanger, and Na⁺-K⁺-ATPase), while their activity is decreased by the stimulation of ET_B-R and dopamine receptors (e.g. D₁-R and D₃-R). ET_B-R, D₃-R, and AT₁-R physically interact with each other. Stimulation of the ET_B-R increases D₃-R expression and function, but the opposite effect occurs with AT₁-R expression and function. The D₃-R also negatively regulates AT₁-R expression. In contrast, stimulation of the AT₁-R increases ET_B-R expression in the long term, and ET_B-R cell surface expression in the short term. Thus, the ET_B-R synergistically interacts with the D₃-R to counterbalance the stimulatory effect of the AT₁-R on sodium transport in the renal proximal tubule.

In immortalized renal proximal tubule cells, D₃ receptor stimulation increases ET_B receptor expression by a calcium-mediated process which is absent in renal proximal tubule cells from spontaneously hypertensive rats [69, 70].

Interaction with the AT₁ Receptor

In the basal state of normal sodium intake and especially during sodium deficit, angiotensin II, via the AT₁ receptor, is pre-eminent in renal sodium conservation. The AT₁ and ET_B receptors physically interact and regulate each other's expression (fig. 1). Long-term activation of the AT₁ receptor increases ET_B receptor expression, whereas short-term activation increases cell surface ET_B receptor expression in rat renal proximal tubule cells, effects that are not observed in renal proximal tubule cells from spontaneously hypertensive rats [71, 72].

Endothelin and Hypertension

Increased ET-1 plasma levels, relative to normotensive subjects, regardless of renal function, have been reported in hypertensive patients in some studies

[73–75]. Most studies, however, have found no differences in circulating ET levels between hypertensive and normotensive subjects [76]. However, the ET_B receptor may be involved in the pathogenesis of hypertension. A high-sodium diet or deoxycorticosterone-salt treatment increases the blood pressure of ET_B receptor-deficient rats. The increased renal sodium transport in these rats probably occurs at a distal tubular level because the increased blood pressure caused by an increased-sodium diet is normalized by blockade of the epithelial sodium channel with amiloride [77]. ET_B receptor blockade produces hypertension that is exaggerated by salt intake or deoxycorticosterone [78, 79]. Systemic ET_B receptor blockade also produces hypertension in mice that is maintained by the ET_A receptor [80]. These findings strongly suggest that the ET_B receptor, by itself or in conjunction with the ET_A receptor, can regulate blood pressure and decreased expression or activity of ET_B receptors increases blood pressure. However, these studies have not determined the involvement of the ET_B receptor expressed in the proximal tubule. As indicated above, the natriuretic effect of ET_B receptor agonists is impaired in spontaneously hypertensive rats. Although ET_B receptor expression is not different in renal proximal tubules between normotensive and spontaneously hypertensive rats, angiotensin II increases total ET_B receptor expression or plasma membrane expression in cells from normotensive, but not hypertensive, rats [71]. The physical interaction between ET_B and AT_1 receptors is also impaired in renal proximal tubule cells from spontaneously hypertensive rats [72]. The blunted natriuretic effect of dopamine in spontaneously hypertensive rats may also be due to an impaired physical interaction between D_3 and ET_B receptors in the proximal tubule [28], resulting in the impaired inhibition of Na^+-K^+ ATPase activity [70].

Conclusion

The renal proximal tubule in all studied species expresses the ET_B receptor. ET has a U-shaped effect on ion and fluid absorption in the renal proximal tubule. The natriuretic effect of ET_B receptor agonists and low nM concentrations of ET is, in part, due to inhibition of sodium and water transport in the proximal tubule. This is due mainly to inhibition of Na^+-K^+ ATPase activity and NHE3 activity, the latter occurring only in the long term, as acute stimulation of ET_B receptors increases NHE3 activity. The inhibition of Na^+-K^+ ATPase activity in renal proximal tubules by stimulation of the ET_B receptor that results in natriuresis is probably abetted by increased positive interaction with the D_3 receptor and negative interaction with the AT_1 receptor. The impaired natriuretic effect of ET_B receptor stimulation in spontaneously hypertensive rats may be caused by impaired ET_B receptor function that may be related to impaired interaction with D_3 and AT_1 receptors.

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