The Intrarenal Endothelin System and Hypertension

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The kidney is both a source of endothelin (ET) generation and an important target organ of this peptide. The highest concentrations of ET-1 in the body exist in the renal medulla, where it mediates natriuretic and diuretic effects through the ETB receptor subtype. It is proposed that aberrations in the renal ET system may lead to sodium and water retention and subsequently to the development of hypertension.

The endothelins (ETs) are a group of vasoconstrictor peptides derived from vascular endothelial cells that act as autocrine/paracrine regulators (9). Three ETs have been identified, ET-1, -2, and -3, all consisting of 21 amino acids. ET peptides are produced within the cells from large precursors, preproendothelins (~200 residues), which undergo two proteolytic cleavages by neutral endopeptidase to form intermediate inactive big ETs (37–39 amino acids). The last step in the biosynthesis of mature/active ET requires the conversion of big ET into ET, which is catalyzed by one or more zinc-binding metalloproteinases, ET-converting enzymes (ECE) (9). Two isoforms of ECE are presently identified: ECE-1 and -2. The former possesses three variants, ECE-1a, -1b, and -1c, all derived from the same gene by alternative splicing (14).

All ECE isoforms are membrane bound, inhibited by phosphoramidon, and able to generate ET-1 from big ET-1. ET-1, the predominant representative of this family, is the most potent natural mammalian vasoconstrictor agent yet discovered (9). The mature ET-1 acts on the cardiovascular system and other target organs by binding to two types of receptors, ETA and ETB. Although ET1 has affinity primarily to ET-1, ETB has equal affinity to all ETs. High abundance of ETA receptors has been detected in the aorta, heart, and kidney, whereas ETB receptors are expressed mainly in the endothelial cells. Activation of ETA receptors on vascular smooth muscle cells (VSMC) increases intracellular calcium levels, leading to prolonged vasoconstriction and cell proliferation. In contrast, activation of ETB receptors, present on endothelial cells, induces the release of nitric oxide (NO) and prostaglandins, thus provoking transient vasodilation (3, 9).

Although it is widely accepted that ETA and ETB receptors mediate vasoconstriction and vasodilation, respectively, several studies have demonstrated that ETB receptors present on VSMC can elicit vasoconstriction. Thus differences in tissue-specific expression and density of the two receptor subtypes, the tissue concentration of the ET, and the preexisting state of the vascular bed determine the type and magnitude of vascular response (vasoconstriction or vasodilation) to this peptide. Plasma levels of ETs are in the picomolar range (1–10 pmol/l), lower than those required to invoke vasoconstriction. However, it should be emphasized that ET-1 is produced in endothelial cells and predominantly secreted toward the adventitial VSMC, supporting the notion that ETs are autocrine/paracrine agents rather than circulating hormones. Nevertheless, the low concentrations of ET in the plasma may permit the efficient clearance of this potentially harmful peptide, mainly by the ETB receptors localized within the lung, heart, and kidney as well as by neutral endopeptidase present in the renal, pulmonary, and vascular tissues.

Although ET-1 is produced predominantly by the endothelial cells, remarkable amounts of this peptide are generated in several tissues/organs such as kidney, heart, brain, and VSMC (3, 8, 9). ET-1 exerts a broad range of actions on these tissues, aimed at modulating blood pressure and controlling extracellular fluid volume. Since the kidney is one of the most important organs in the regulation of systemic hemodynamics, it comprises a central target organ for ET-1 as well as a major site of production of this peptide.

The renal ET system

In the last decade, a large body of evidence has accumulated indicating that locally produced vasoactive substances, including ET-1, play an important role in the regulation of kidney hemodynamics and its excretory function. The renal medulla is an important site of generation of ET-1 and actually contains the highest concentrations of immunoreactive ET-1 (irET-1) in the body (8, 10). Likewise, remarkable amounts of ET-1 are detectable in most renal cell types along the nephrovascular unit, where they act in proximity to their production site. ET-1 affects three aspects of renal function: 1) hemodynamics of the kidney, 2) tubular handling of electrolytes and water, and 3) proliferation and mitogenesis of certain renal cell types such as mesangial cells and VSMC.

Concerning the first aspect, the renal vasculature is highly sensitive to ET-1 activity compared with other vascular beds. ET-1 is the most potent known renal vasoconstrictor agent, 30–50 times more potent than well-known vasoconstrictors such as norepinephrine and angiotensin II. Systemic infusion of ET markedly decreases renal blood flow (RBF) as a result of a profound and sustained increase in renal vascular resistance. The sustained renal vasoconstriction results from constriction of the
glomerular afferent and efferent arterioles as well as the arcuate and interlobular arterioles. The sustained renal vasoconstriction is often preceded by a transient vasodilatory response, possibly due to ETB receptor-mediated release of NO (3, 8). Glomerular filtration rate (GFR) is also decreased in response to systemic infusion of ET. Similarly, short-term infusion of ET-1 into the renal artery decreases renal plasma flow (RPF), GFR, and urinary flow rate (8). Long-term infusion of ET-1 into conscious dogs resulted in increased renal vascular resistance and decreased renal perfusion/filtration (7, 8). In most clearance studies, the adverse hemodynamic effects of ET-1 were associated with decreased sodium excretion, which was attributed to the high doses of ET-1. A systemic infusion of ET-1 at high doses resulted in a profound antiuretic and antinatriuretic response, apparently secondary to the decreased RBF and GFR. However, when ET-1 was administered at low doses it induced diuretic and natriuretic effects (8).

These dose-dependent differences in the renal response to ET-1 have led to the widely accepted notion that the excretory effects of low doses of ET-1 truly represent the intrinsic de novo actions of locally produced ET-1. The nephron-derived ET-1 inhibits Na⁺-K⁺-ATPase activity and directly blocks the stimulatory effects of vasopressin on water reabsorption in the collecting duct (Fig. 1). Indirect evidence supporting the regulatory role of tubular ET-1 in sodium and water transport has emerged from several studies demonstrating that, in contrast to ET-1, administration of the ET-1 precursor big ET-1 causes diuresis and natriuresis in association with a marked increase in blood pressure (4) (Figs. 2 and 3). Most likely, local conversion of big ET-1 by ECE allows the mature peptide to gain access to renal sites not accessible to exogenous ET-1. Thus it has been claimed that the hemodynamic and excretory actions of big ET-1 reflect the renal effects of de novo-produced ET-1.

In this context, accumulating evidence suggests that the stimulatory effects of ET-1 on water and sodium excretion are mediated through ETB receptors. First, pretreatment of rats with A-192621.1, a highly selective ETB antagonist, significantly abolishes the diuretic and natriuretic responses induced by big ET-1 (4) (Fig. 2). This finding is in line with several reports that demonstrated high abundance of ETB receptors in the renal medullary collecting duct epithelium, the main inhibitory site of ET-1 action on sodium and water reabsorption. Second, activation of ETB receptors stimulates the release of NO, which plays a crucial role in the regulation of renal hemodynamics and excretory function (1). Indeed, inhibition of NO production coupled to ETB activation has been shown to reduce sodium excretion and suppress the pressure-natriuresis response and the diuretic and natriuretic responses induced by big ET-1 (Fig. 3). Moreover, it has been shown that ET-1 acts through ETB receptors to induce transient medullary vasodilation, which may contribute to the diuretic/natriuretic actions of locally produced ET-1 in the renal medulla (1). Since the renal ET system may contribute to the body fluid volume and electrolyte balance, it has been suggested that perturbation in this system may lead to some forms of hypertension.

Renal ET and hypertension

Independent of its direct effect on the vasculature that contributes to the regulation of vascular tone and blood pressure, ET may affect blood pressure indirectly by modulating renal hemodynamics and excretory functions of the kidney. The kil-
ney expresses both ETA and ETB receptor subtypes. In humans, ETA is expressed primarily in the renal vasculature, whereas ETB receptors are of particular abundance in the epithelial cells of the inner renal medullary collecting duct (8). As in peripheral blood vessels, ETA in renal vasculature mediates the vasoconstrictive action of ET-1 of local or systemic origin, causing decreased RBF. In contrast, the involvement of ETB receptors in the regulation of vascular tone is attributable to their pivotal role in mediating the stimulatory effects of locally produced ET (mainly in the medulla) on sodium and water excretion. Therefore, intact ETB function in the medullary tissue appears to mediate the inhibitory actions of ET-1 on sodium and water reabsorption, leading to natriuresis and diuresis, thus reducing blood pressure.

On the other hand, interference with the medullary ETB receptors may lead to sodium and water retention and, thereby, to hypertension. It is widely accepted that ET-1 derived from the endothelial cells of the renal vasculature or from the circulation provokes vasoconstriction, thereby reducing both RBF and GFR and subsequently diminishing sodium and water excretion. On the contrary, however, locally produced ET-1 in the medulla provokes vasodilation and promotes salt and water excretion via ETB receptors. It should be emphasized that the involvement of renal ETB in the development of hypertension is totally different from that of the renal ETB receptor subtype and therefore will be discussed separately.

Several studies have shown that the ET system is activated in different models of salt-sensitive hypertension: deoxycorticosterone acetate (DOCA)-salt-hypertensive or DOCA-salt-treated spontaneously hypertensive rats (SHR), Dahl salt-sensitive rats, stroke-prone SHR, and fructose-fed or angiotensin II-infused hypertensive rats (12). Although most of these studies examined and documented overexpression of ET-1 in the peripheral vasculature or the cardiac tissue, a few studies have shown increased expression of ET-1 in the vasculature and glomeruli of the kidney in some of these models, namely DOCA-salt-hypertensive rats (12). Enhanced synthesis of this peptide, especially by the cortical renal vasculature in proximity to ETA, may result in renal vasoconstriction, which is known to influence systemic blood pressure. However, other studies failed to demonstrate increased synthesis of ET-1 in the renal tissue and some of them even observed decreased production of this peptide (10, 12).

It should be noted that similar patterns of plasma ET-1 levels have been found in patients and animals with hypertension. Despite the importance of plasma and renal endogenous levels of ET-1, differences in the vasculature sensitivity between normotensive subjects/animals and hypertensive ones may result in enhanced activity of ET-1, regardless of its concentration. For example, there are several reports that demonstrated enhanced renal vascular responsiveness to ET-1 in SHR (10, 12). The enhanced sensitivity of the renal vasculature to ET-1 may stem from increased ETA receptor expression, which was indeed documented in the VSMC of the aorta and mesenteric arteries of DOCA-hypertensive rats. Likewise, decreased renal clearance of ET-1 has been implicated in the hypersensitivity of DOCA-salt-sensitive rats to administered ET-1 (10). Together, these data do not provide firm evidence supporting either of the above-mentioned possibilities.

Fortunately, the development of numerous ET-1 antagonists in recent years has led to renewed interest in this field and has led to the development of specific antagonists for ETA and ETB receptors.
provided excellent tools to address the involvement of the renal ET system in the pathogenesis of hypertension. In this context, one of the earliest studies, conducted by Ohno et al. (11), demonstrated that infusion of antibodies to ET-1 into SHR resulted in increased GFR and RBF in association with a decrease in blood pressure. No effect was observed in Wistar-Kyoto (WKY) control rats that underwent a similar procedure. A similar improvement in kidney function was achieved in SHR and DOCA-salt-hypertensive rats, but not in the relevant controls, when the ET-1 was blocked by various ETα antagonists (10, 12). These findings are in line with the notion that enhanced ET-1 vasoconstriction in certain animals with genetic hypertension may be involved in the development of hypertension. However, these studies should be considered carefully, since both the obtained improvement in renal function and the lowering of blood pressure may be secondary to blockade of circulatory ET-1 and not necessarily to the inhibition of renal ET-1.

In contrast to the serious doubts raised about the involvement of the renal vasculature response to ET-1 in the pathogenesis of hypertension, several studies have provided evidence that implicates the locally produced ET-1 in the medulla in the development of experimental and clinical hypertension. One of the earliest observations in this regard was that of Hoffman et al. (5), who demonstrated that the mean daily urinary excretion of irET-1 in hypertensive patients was significantly lower than in normotensive subjects (Fig. 4). Clearance studies revealed that urinary ET-1 is of renal origin; therefore, the impaired ET-1 excretion in these patients reflected reduced capacity of the kidney (mainly the medullary tissue) to synthesize ET-1. When the hypertensive patients were fed a high-salt diet, the salt-sensitive subgroup excreted less ET-1 than the salt-resistant hypertensive subgroup (5). These authors suggested that, since ET-1 has diuretic and natriuretic properties when produced locally, the decreased renal excretion of ET-1 might be of relevance to the pathophysiology of hypertension and salt sensitivity.

A similar finding was obtained in SHR, where urinary ET-1 excretion was significantly lower than that measured in WKY rats (6). Interestingly, these differences were observed in 8- to 9-wk-old rats (the age of development of hypertension), but not in 3- to 4-wk-old rats, which have not yet developed elevated blood pressure (6). However, other authors have found a small, nonsignificant difference in the urinary excretion of ET-1 between SHR and normotensive controls. Most recently, Vogel et al. (15) found no difference in the urinary excretion of ET-1 between Prague hypertensive rats (PHR) and normotensive counterparts (PNR). Quantitative RT-PCR revealed comparable levels of ET-1 mRNA in the renal cortex and outer medulla of PHR compared with PNR, whereas the renal papillae from PHR showed a 58% decrease in PHR compared with their normotensive counterparts. ELISA demonstrated a similar decrease in irET-1 in the papillae but not in the cortex and outer medulla of PHR. These findings are in agreement with the majority of studies, which clearly showed reduced levels of ET-1 in the renal medulla but not in the cortex of different models of hypertensive rats.

However, these authors went on to examine the expression and distribution of an additional member of the ET system, ET-3, which is known to be preferentially expressed in the renal medulla and to induce natriuretic and diuretic responses. Surprisingly, the expression of ET-3 and immunoreactive peptide was significantly higher in the outer medulla of PHR compared with PNR but was similar in the papillae of both subgroups. No differences between PHR and PNR were found in the density of ETα and ETβ (15). These data suggest that decreased synthesis of ET-1 in the renal medulla may contribute to water and salt reabsorption at the IMCD and thus may allow the development of hypertension in this model. The physiological relevance of the high levels of ET-3 in the papillae of PHR awaits further investigation, although it may represent a compensatory response aimed at counterbalancing the consequences of the decreased levels of ET-1.

The impairment of intrarenal ET-1 system efficacy is unique in this regard, since, as mentioned before, enhanced produc-
tion of ET-1 has been observed in several experimental models of sodium-dependent hypertension such as DOCA-salt-treated normal rats, SHR, and Dahl salt-sensitive rats (11). This enhancement in ET-1 expression/generation was demonstrated in the blood vessel wall, circulation, and even in the urinary excretion rate of this peptide in uninephrectomized DOCA-treated rats given excess salt. However, most of these studies have suggested that the increased renal synthesis of ET-1 is aimed at restoring sodium excretion (3, 8, 9). Therefore, these observations are not at odds with our concept that impairment of ET-1 production in the renal medulla of hypertensive patients could lead to the development of high blood pressure. It should be emphasized that not all of the studies have reported the activation of the ET-1 system in the blood vessels or at the circulatory level of patients with hypertension, and some studies failed to show correlation between the urinary ET-1 excretion and blood pressure (3, 10, 12). Nevertheless, many of the studies that addressed this issue have demonstrated a strong correlation between the urinary ET-1 and salt excretion, and that relationship is influenced by hypertension, suggesting that the excretion of ET-1 may be a useful biological marker for the prediction of salt-sensitive hypertension.

An additional approach that has been developed in recent years to shed light on the involvement of the renal ET system in the pathogenesis of hypertension is gene targeting strategies of a key component of this system, i.e., the ETB receptor subtype. In this regard, rats with naturally occurring or targeted mutation of ETB exhibit coat color spotting and intestinal aganglionicosis (Hirschsprung disease) (2). Rats homozygous for this mutation (ETB<sup>sl/sl</sup>) die shortly after birth because of intestinal obstruction. To investigate the physiological and pathophysiological roles of ETB receptors in these rats, Gariepy et al. (2) used a new technique: tissue-specific transgenic rescue of the intestinal phenotype by human dopamine hydroxylase (DBH) promoter, leaving the renal ETB mutation intact. Interestingly, DBH-ETB<sup>sl/sl</sup> rats exhibited normal arterial blood pressure when placed on a sodium-deficient diet, but on a high-sodium diet they developed severe hypertension. Treatment of these rats with ET<sub>A</sub> antagonist significantly decreased blood pressure. Complete restoration of blood pressure to normal levels was achieved by blocking the epithelial sodium channel with amiloride. The authors concluded that DBH-ETB<sup>sl/sl</sup> rats are hypertensive because they lack the normal tonic inhibition of these sodium channels because of a lack of ETB receptors or reduced levels of the de novo ETB agonist, i.e., ET-1. These findings explain the “paradoxical” results of the initial studies, which demonstrated that heterozygous ET<sup>−/−</sup> mice displayed increased blood pressure despite their reduced plasma and lung tissue levels of the mature ET-1. The common denominator of all of these studies is an association between reduced renal ET-1 generation or impaired abundance of ETB receptors in the medullary tissue and elevated blood pressure. Therefore, it is conceivable that defects in the renal ET system cause sodium and water retention as well as salt sensitivity and thereby volume-overload hypertension (Fig. 1). Since activation of ETB receptors leads to a variety of intracellular events, one of which is the release of NO metabolites (NO<sub>2</sub> + NO<sub>3</sub>) in the urine of hypertensive patients. Indeed, Sierra et al. (13) demonstrated that urinary excretion of NO<sub>2</sub> + NO<sub>3</sub> was lower in most hypertensive patients, especially those with high renal vascular resistance, than in normotensive subjects. Since NO plays an important role in the regulation of medullary blood flow (1), impairment of NO production may reduce intrarenal blood flow and subsequently reset the pressure-diuresis relationship, leading to prompt sodium retention and contributing to high blood pressure. Also, animals suffering from experimental hypertension, such as SHR, Dahl salt-sensitive rats, and DOCA-salt-hypertensive rats, exhibit abnormal endothelial function.

Together, these findings strongly suggest that tonic activation of NO-coupled ETB receptors in the renal medulla causes natriuresis and diuresis and thus tends to decrease blood pressure. Interference with the medullary ET system, by reducing either ET-1 content or ETB expression, diminishes salt and water excretion, leading to salt-dependent hypertension.

References