REVIEW

Role of Endothelin in the Pathogenesis of Hypertension

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In 1985, investigators characterized a potent vasoconstrictor of endothelial origin called endothelin (ET). Subsequently, 3 peptides were recognized that had a comparable molecular structure but different receptors that mediate potent vasoconstrictive and mild vasodilative effects. The renal effects are characterized by natriuresis despite renal vasoconstriction. This effect, along with the stimulation of ET by high sodium intake, suggests that ET may be responsible for maintaining sodium balance when the renin-angiotensin system is depressed. Endothelin is activated in desoxycorticosterone acetate salt hypertension models and salt-sensitive hypertension. However, ET involvement with spontaneous hypertension models and renovascular hypertension in rats appears minimal. In humans, the role of ET appears similar to that in experimental animals; in both, ET regulates salt metabolism. Salt-sensitive patients exhibit a blunted renal ET-1 response during sodium load. The role of ET in humans has been investigated using nonspecific ET receptor blockers that inhibit the vasoconstrictive and vasodilative components of ET. However, the effects of ET blockade should be investigated with ET subtype A receptor blockers that mediate vasoconstriction alone. Effects of ET blockade also should be evaluated with respect to stimulation of oxidative stress and tissue damage, important mechanisms responsible for tissue fibrosis. This review offers the clinician a balanced view on the hypertensive mechanisms involved with activation of ET and associated clinical implications.

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Ang II = angiotensin II; AVP = arginine vasopressin; DOCA = desoxycorticosterone acetate; DS = Dahl salt-sensitive; ECE = ET-converting enzyme; ET = endothelin; GFR = glomerular filtration rate; IMCD = inner medullary collecting duct; IP_3 = inositol triphosphate; K_f = ultrafiltration coefficient; 2K-1C GH = 2-kidney 1-clip Goldblatt hypertension; MAP = mean arterial pressure; mRNA = messenger RNA; NEP = neutral endopeptidase; NO = nitric oxide; PKC = protein kinase C; P_{uf} = net filtration pressure; RBF = renal blood flow; SHR = spontaneously hypertensive rat; VSMC = vascular smooth muscle cell

The endothelium has been recognized as an extremely active source of vasoactive substances and a major regulator of vascular tone. Most studies have focused on endothelial-derived relaxing factors; however, the endothelium also produces several vasoconstrictors. One such vasoconstrictor was described initially by Hickey et al.[1] Subsequently, in the supernatant of cultured porcine aortic endothelial cells, Yanagisawa et al[2] isolated and purified a 21–amino acid endothelial vasoconstrictor peptide that they named endothelin (ET). Three isoforms of ETs in humans, namely ET-1, ET-2, and ET-3 (Figure 1), interact with 2 distinct subtype receptors, ET_A and ET_B, to exert their biological effects. Since the discovery of ETs, the diverse roles that this family of peptides plays in numerous physiological and pathophysiological conditions have been revealed, leading to the recent development of therapeutic agents that inhibit the formation of ETs either by blocking ET-converting enzyme (ECE) or by receptor blockade. Such antagonists may prove useful in the treatment of various cardiovascular and renal diseases. This overview describes the cardiovascular and renal physiological effects of ET-1 and the probable role of ET-1 in hypertension.

THE ET FAMILY

All ETs consist of 21 amino acids; ET-1 and ET-2 are extremely similar, whereas ET-3 differs from ET-1 at 6 of 21 positions. As shown in Figure 1, the ET isopeptides share a marked structural similarity to sarafotoxin, a peptide isolated from the Israeli burrowing asp (*Atractaspis engaddensis*).[3] The ET isoforms are encoded by 3 independent genes located on chromosomes 6, 1, and 20, respectively.[4] Expression of the ET genes is located in several organs and tissues, including the kidneys, brain, lungs, gut, and endothelium. The ET peptides are produced within the cells from large precursors named preproendothelins that possess approximately 200 amino acids (Figure 2). The metabolic pathway for biological activation is similar for the 3 ETs. Human ET-1 messenger RNA (mRNA) encodes 212 amino acids that undergo 2 proteolytic cleavages by a neutral dibasic-pair–specific endopeptidase and a carboxypeptidase to form big ET-1, a 39–amino acid propeptide (Figure 2). Big ET-1, which is inactive, is converted both inside and outside the cell to ET-1 by ECEs, a family of metalloproteases.[5]

THE ECEs

To date, 7 isoforms of ECEs have been identified: ECE-1a, ECE-1b, ECE-1c, ECE-1d, ECE-2a, ECE-2b, and ECE-3.[6]

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These isoforms are serine, aspartate, or soluble thiol proteases. The ECE-1 and ECE-2 isoforms, apparently the most important, are membrane metalloproteases that are inhibited by phosphoramidon. Both selectively cleave the tryptophan-valine bond in the carboxy terminal portion of big ET-1. Endothelin-converting enzyme 1 is localized in the plasma cell membrane of several tissues, and its optimal activity is at pH 7.0.[7] These ECEs also hydrolyze bradykinin, substance P, and insulin. Endothelin-converting enzyme 2 is localized in the trans-Golgi network, and its optimal activity is at pH 5.5; it is expressed most abundantly in neural tissues. These zinc-dependent endoproteases may mediate the vasoconstrictive effects of big ET-1 in the vascular system. The human ECE-1 shares 37% amino acid sequence identity with neutral endopeptidase (NEP) 24.11.[8] Endothelin-converting enzyme 1, NEP, and angiotensin-converting enzymes are zinc metalloproteases whose activities can be inhibited simultaneously by a common zinc-binding group such as phosphoramidon, a sulfhydryl, a hydroxamic acid, or a carboxylic group. Other enzymes are also capable of cleaving big ET-1 into biologically active peptides. For instance, chymase in airway mast cells can cleave big ET-1. However, this cleavage is at the tyrosine 31–glycine 32 peptide bond, yielding ET-1 (1-31 amino acids), which constricts the smooth muscle cells of the trachea and may be involved in allergic inflammation.[9] In addition, matrix metalloproteinase 2, which plays a role in physiological vascular remodeling, tissue repair, and angiogenesis, cleaves specifically the glycine 32–leucine 33 bond of big ET-1 to form ET-1 (1-32 amino acids), which induces vasoconstriction of the mesenteric arteries. In contrast, ECEs are expressed mainly by the vascular endothelium; their vasoconstrictive effect is even more powerful than that of ET-1 (1-21 amino acids). Not all the enzymes responsible for the final step of posttranslational processing of ET-1 have been identified. However, proteases besides ECE-1 and ECE-2 are involved in the synthesis of ET-1.

ET SECRETION

Once ET-1 has been formed, it is secreted primarily via a constitutive pathway, although a regulated pathway via secretory granules also has been described. Endothelin 1 is secreted predominantly by vascular endothelial cells to act on the underlying smooth muscle cells; however, its secretion also has been shown in several other cells and tissues.[10-15] Because ET secretion is primarily via a constitutive pathway, regulation of ET-1 secretion primarily entails changes in gene expression. Indeed, several regulatory elements are found in the 5′ region of the *ET-1* gene.[4] These characteristics of the *ET-1* gene are similar to those of the so-called early response genes characterized by rapid upregulation elicited after a change. Because of this type of regulation and the fact that ET-1 is secreted primarily toward the basolateral side of the cell, plasma levels of ET-1 change slowly in response to diverse stimuli. Hence, induction of ET secretion above basal levels requires 2 to 5 hours.[14,15] Endothelin 1 plasma levels usually are seen in only the picomolar range (1-10 pmol/L),[15] lower than those required to invoke vasoconstriction. Considering that approximately 80% of the total amount of ET-1 synthesized by endothelial cells is released toward the basolateral side of cells, tissue levels are likely higher than plasma levels. Thus, ET-1 probably acts primarily as a paracrine/autocrine mediator and not as a circulating hormone.[16]

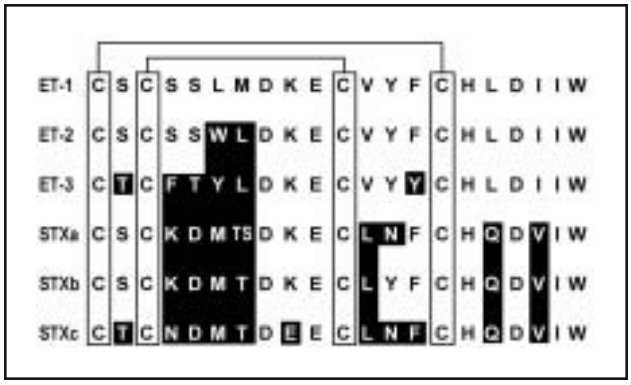

FIGURE 1. Structure of endothelins (ETs) and sarafotoxins (STXs). All native endothelins and sarafotoxins have 2 intramolecular disulfide bonds (indicated by horizontal brackets at the top of the figure). Amino acids enclosed in boxes indicate major differences in molecular structure among the 6 compounds. From *Circulation.* 1991;84: 1457-1468, with permission.

Several factors can stimulate or inhibit ET secretion. The possible importance of all the naturally occurring humoral substances that affect the release of ET is beyond the scope of this review. Most of the factors that influence ET release are listed in Table 1. Vasoconstrictors, such as norepinephrine,[2] angiotensin II (Ang II),[17] vasopressin,[18] F_2-isoprostane,[19] serotonin,[20] oxidized low-density lipoprotein,[21] and transforming growth factor β,[22] stimulate the synthesis and/or the release of ET. Vasodilators, such as bradykinin,[23] nitric oxide (NO),[24] prostaglandins E_2 and I_2,[25] atrial and brain natriuretic factors,[26] and adrenomedullin,[27] often inhibit the release of ET. Within this conceptual framework, the interaction between ET and the renin-angiotensin system deserves to be examined because of its important role in hypertension. In several studies, Ortiz et al[28,29] showed that sustained hypertension induced by subpressor doses of Ang II (which do not produce immediate elevation of blood pressure) stimulates oxidative stress with subsequent stimulation of ET. The oxidative stress–

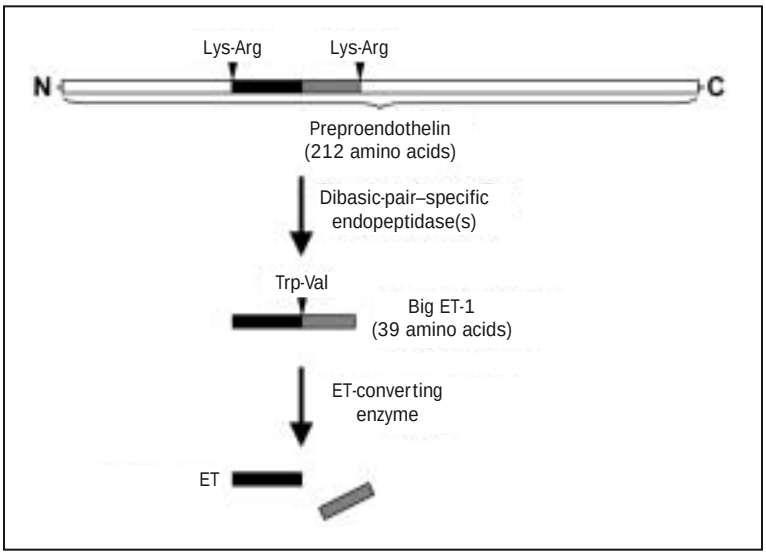

FIGURE 2. Biosynthesis of endothelins (ETs). Preproendothelin is synthetized containing 212 amino acids. This large molecule is hydrolyzed by a dibasic-pair–specific lysine-arginine (Lys-Arg) endopeptidase forming big ET-1 (39 amino acids). Finally, an ET-converting enzyme splits big ET-1 at tryptophan-valine (Trp-Val) binding, releasing ET (21 amino acids). From *Circulation.* 1991;84:1457-1468, with permission.

induced increase in ET was interpreted to be responsible for the elevation of blood pressure. In fact, blood pressure was decreased either by use of an antioxidant (tempol) or by blockade of ET receptors. These studies suggested that the activation of ET was oxidative stress dependent because antioxidant use was followed by a decrease in plasma ET, but the blockade of ET receptors did not alter the level of oxidative stress. Consistent with the stimulatory effect that Ang II exerts on ET are the inhibitory effects of ET on renin release, which could be interpreted as modulatory feedback. In this manner, the increase in blood pressure initiated by Ang II could be sustained by oxidative stress and ET because renin release would decrease progressively.

To define the physiological role of ET, attention has been focused on biophysical factors (Table 1). Changes in the release of ET induced by mechanical strain[30,31] and/or hemodynamic hydrostatic pressure should be interpreted with caution because the same physical factors also affect the release of vasodilators such as NO, prostaglandins, etc. Whether changes in the release of ET, produced by changes in shear stress, have some physiological importance is unknown.[30,31] Systemically, an increase in intra-arterial pressure greater than 70 to 75 mm Hg produces a progressive increase in arterial vasoconstriction that maintains constant blood flow to the organs.[32] This phenomenon of blood flow autoregulation is mediated by a myogenic response produced by changes in cellular calcium due to activation of calcium channels sensitive to arterial wall tension.[32] However, determining the exact role of ET in modulating such a response requires more in vivo studies.

RECEPTORS AND SIGNAL TRANSDUCTION

Endothelins exert their actions via 2 receptor subtypes, ET_A and ET_B,[33-35] which belong to the super-family of G protein–coupled receptors. The human ET_A receptor, believed to be involved in vasoconstrictive and proliferative responses to ET-1,[14,16] contains 427 amino acids and binds with the following affinity: ET-1 > ET-2 > ET-3.[33,34] The human ET_B receptor contains 442 amino acids and binds all ETs with equal affinity; its activation induces transient vasodilation. When ET-1 binds to its receptors, it elicits unusually prolonged biological effects because of the almost irreversible binding of the peptide to its receptor, shown by the fact that ET-1 remains associated with the ET_A receptor up to 2 hours after endocytosis.[34] The distribution of ET receptors differs among the different tissues.[33] In vascular tissue, ET_A receptor mRNA is expressed predominantly in smooth muscle,[35] whereas ET_B receptor mRNA is most abundant in endothelial cells. This suggests that constriction of vascular smooth muscle is likely mediated by ET_A receptors, whereas ET_B receptors decrease vasoconstriction by release of endothelial-relaxing factors.[33] Endothelin B receptors also are expressed in other human cells, such as vascular smooth muscle cells (VSMCs),[36,37] macrophages, and platelets[38]; ET_A and ET_B

TABLE 1. **Factors That Influence ET-1 Secretion***

Stimulating factors	Inhibiting factors
Vasoconstrictor	Vasodilators
Angiotensin II	Bradykinin
Vasopressin	Nitric oxide
Norepinephrine	Prostaglandins E_2 and I_2
Isoprostane 8-epi-prostaglandin $F_{2\alpha}$	Adrenomedullin
	Atrial and brain natriuretic peptides
Thrombogenic agents	Anticoagulants
Thrombin	Heparin
	Hirudin
Cytokines and growth factors	NA
Interleukin 1 and 3	
Tumor necrosis factor α	
GCSF	
Interferon-gamma	
Transforming growth factor β	
Endotoxin	
Physicochemical factors	Physicochemical factors
Mechanical strain	High levels of shear stress (>6 dyne/cm^2)
Pressure without cell distortion	NA
Hypoxia	NA
Low levels of shear stress (<2 dyne/cm^2)	NA
Hemodynamic pressure overload	NA
Aging	
Other factors	Other factors
Insulin	Nitrates
Serotonin	Progesterone
Corticosteroids	Estrogens
Erythropoietin	PPAR-α
Oxidized low-density lipoproteins	Calcium ionophores
Cyclosporine	Montelukast
Platelet aggregation	NA
Macrophage infiltration	NA
Formation of atherosclerotic lesions	NA

*ET-1 = endothelin 1; GCSF = granulocyte-macrophage colony-stimulating factor; NA = not applicable; PPAR-α = peroxisome proliferator-activated receptor-α.

receptors are particularly abundant in the kidneys.[39] The membrane transduction process that leads to a vasoactive response (Figure 3) includes a G protein–coupled cell surface receptor, coupling G protein and phospholipase C–protein kinase C (PKC) pathway or other G protein–activated effectors. The ET_A-induced activation of phospholipase C leads to the formation of inositol triphosphate (IP_3) and diacylglycerol from phosphatidylinositol. The IP_3 then diffuses to specific receptors on the endoplasmic reticulum and releases stored Ca^{2+} into the cytosol. This causes a rapid but short-lived (seconds) elevation in intracellular Ca^{2+}.

Binding of ET-1 to ET_A receptors also activates receptor-operated and voltage-gated calcium channels in the plasma membrane, allowing entry of extracellular calcium into the cell. This last effect of ET-1, mediated by IP_3 and/or PKC, results in a sustained (minutes) elevation in intracellular calcium, which contributes to the prolonged smooth muscle cell contraction elicited by the peptide. Endothelin B coupling activates soluble phospholipase A_2 and guanylate cyclase, leading to an increase in intracellular cyclic guanosine monophosphate. This change stimulates the release of NO and prostaglandin I_2, producing vasodilation. Furthermore, ET-1 increases expression of transcription factors, such as c-*fos*, c-*jun*, c-*myc*, and VL-30.[40] Endothelin isopeptides, through either ET_A or ET_B receptors, also induce the mitogen-activated protein kinase cascade[16] that may mediate the long-term action of ET.

DEGRADATION AND CLEARANCE OF ET

Plasma ET reflects spillover from local release of ET-1. However, ET-1 that reaches the circulation is removed rapidly with a plasma half-life of less than 1.5 minutes. It is cleared from the circulation primarily by the renal and splanchnic circulations via several ET-1–degrading proteases, including NEPs.[41] Leukocytes also can inactivate ET via enzymes, such as cathepsin G, during inflammatory states in the vascular endothelial bed.[42] The lung has a more unique way of clearing ET. Circulating ET-1 binds to ET_B, which is then internalized and degraded.

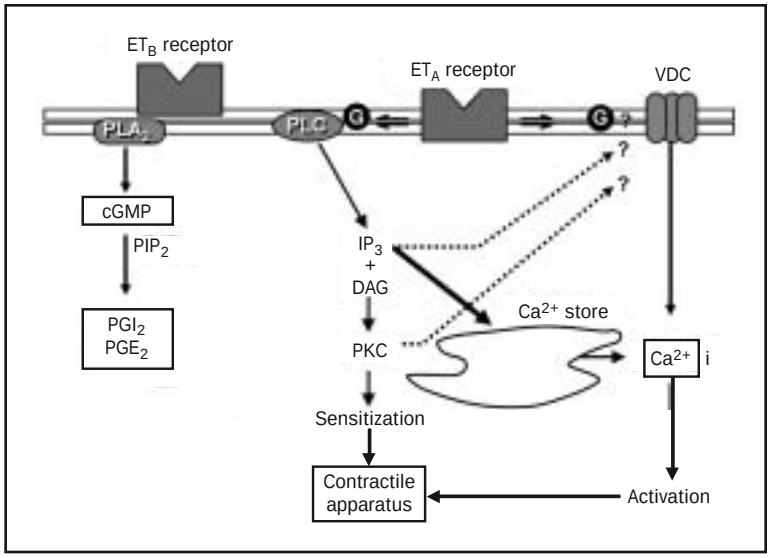

FIGURE 3. Endothelin (ET) receptors and signal transduction. The ET_A receptor activates transduction mediators that promote vasoconstriction. Stimulation of phospholipase C (PLC) hydrolyzes diacylglycerol (DAG) and inositol triphosphate (IP_3), which releases Ca^{2+} from its intracellular (i) stores. cGMP = cyclic guanosine monophosphate; G = G protein; PGE_2 = prostaglandin E_2; PGI_2 = prostaglandin I_2; PIP_2 = phosphatidylinositol; PKC = protein kinase C; PLA_2 = phospholipase A_2; VDC = voltage-dependent calcium channels. Adapted from *Circulation.* 1991;84:1457-1468, with permission.

CARDIOVASCULAR AND HEMODYNAMIC EFFECTS OF ET

Intravenous infusion of ET-1 into conscious rats causes an initial decrease in blood pressure that is followed by intense and prolonged (several hours) hypertension.[13,15,16] The initial decrease in blood pressure apparently is due to activation of the ET_B receptor, which in turn increases release of NO and prostacyclin from the endothelium, atrial natriuretic peptide, and adrenomedullin.[43,44] The subsequent vasoconstrictive response is due to the direct action of ET-1 on the smooth muscle cells via the ET_A receptor[45,46] and exceeds the vasoconstrictive potency of Ang II or catecholamines. The prolonged vasoconstriction is not dependent on the plasma levels of ET-1, but rather on the slow dissociation from its receptors. As with ET infusion, increasing endogenous ET-1 levels (via viral transfer of human preproendothelin 1 complementary DNA into rat liver, which increases plasma ET-1 levels 6-fold) also increases blood pressure.[47] These hypertensive effects of ET may be due to its direct vasoconstrictive properties, as well as to the ET-1 amplification of the response to other vasoactive agents, including norepinephrine and serotonin.[45] Similarly, these agents potentiate the vasoconstrictive effect of ET-1.[48] Blocking the effects of endogenous ET by using a receptor antagonist or by genetically disabling the ET system provides further evidence that ET is involved in regulating vascular tone. For instance, systemic administration of a nonspecific ET_A receptor antagonist into healthy individuals causes an increase in forearm blood flow and a small reduction in blood pressure,[46] suggesting that endogenous ET may increase basal vascular tone. However, studies that have genetically manipulated the ET system suggest that the role of endogenous ET may be more complex. In fact, studies in homozygous ECE-1 gene knock-out mice (in which the *ET-1* gene has been deleted) showed a paradoxical slight elevation of blood pressure,[49] suggesting the absence of a predominant vasodilative action of endogenous ET-1. However, this experimental model has severe craniofacial and cardiac abnormalities, making it difficult to rule out anatomical abnormalities that could interfere with normal regulation of organ blood flow. Interestingly, these abnormalities are virtually identical to those observed in ET-1 or ET_A receptor–deficient animals and have a developmental phenotype similar to that observed in the ET-3 or ET_B receptor gene knock-out mice.[50] Studies of backcross inbreeding effects between heterozygous mice for targeted disruption of the ET_B receptor with mice homozygous for the piebald mutation of the ET_B gene showed that the progeny of such mice, with only $^1/_8$ levels of ET_B receptors, exhibited elevated blood pressure.[50] When these mice were treated with the ET_B receptor antagonist BQ-788, blood pressure increased in those

progeny with at least 1/5 levels of ET_B receptors and in the wild type with high ET_B levels, but not in the progeny with 1/8 levels. Together, these results suggest that the ET family plays a hypotensive role via the ET_B endothelial receptor.[50]

CARDIAC EFFECTS OF ET

In the heart, ET affects the coronary circulation, the cardiac myocytes, and the conduction system. Endothelin 1 produces vasoconstrictive responses in human coronary circulation and may play a role in the etiology of coronary vasospasm.[51-53] Furthermore, ET-1 directly enhances platelet aggregation and thrombus formation, which in turn aggravate coronary atherosclerosis and coronary ischemia.[51,53,54] Endothelin 1 also is a potent mitogen of cardiac myocytes, increasing the release of natriuretic peptides, and has a positive inotropic effect.[55] In fact, increased vascular ET-1 production is associated with remodeling and hypertrophy[56,57] and may contribute to the Frank-Starling response and hypertrophy in rat hearts.[58] Finally, when administered directly into coronary vessels of experimental animals, ET-1 causes arrhythmias, including ventricular fibrillation,[59] attributed to a prolongation of action potential and development of after-depolarization potentials.[60]

RENAL EFFECTS OF ET-1

Human kidneys not only have the capacity to produce ET but also contain a high concentration of receptors. In fact, the renal medulla contains the highest concentration of receptors in the body,[61,62] with type B receptors making up 70% of the receptors in both the cortex and medulla.[63] These receptors are located in the vasculature, renal tubules, mesangial cells, and medullary interstitium.[64] Thus, it is not surprising that ET plays a major role in regulating renal hemodynamics, tubular handling of water and electrolytes, and proliferation and mitogenesis of mesangial cells and VSMCs. For simplicity, we break down the renal effects of ET into hemodynamic and tubular effects.

Renal Hemodynamic Effects

Endothelin 1 is widely accepted as the most powerful known renal vasoconstrictive agent, 30 to 50 times more potent than equimolar quantities of norepinephrine and Ang II. Of note, however, the effects of ETs differ depending on the dose and whether ETs are infused into the systemic circulation or directly into the renal circulation. Systemic infusion of high doses of ET-1 causes a marked increase in renal vascular resistance, a decline in renal blood flow (RBF) and glomerular filtration rate (GFR), and a marked decrease in renal perfusion/filtration accompanied by antidiuresis and antinatriuresis.[65] Changes in RBF occur rapidly and are dose dependent. Direct infusion of ET-1 into the renal artery results in an immediate transient increase in RBF, followed by a marked prolonged decrease in both RBF and GFR. In the glomerulus, ET-1 acts on both variables that determine GFR: the net filtration pressure (P_{uf}) and the ultrafiltration coefficient (K_f). Infusion of mildly pressor doses of ET-1 causes a greater increase in efferent than in afferent arteriolar contraction, which tends to augment P_{uf}. However, ET-1 causes a decline in K_f (by inducing mesangial cell contraction) and a modest reduction in glomerular capillary flow rate. These combined effects cause GFR to remain relatively constant.[64,65] Higher doses of ET-1 produce an increase in afferent and efferent arteriolar resistance and a decline in glomerular capillary flow rate and K_f, thereby reducing GFR.

These studies reveal the pharmacological effects of ET; however, they are not representative of the in vivo physiological actions because plasma levels of ET are usually extremely low, and ET release by endothelial cells is polarized toward the abluminal side. To elucidate the physiological role of ETs, several investigators have examined the systemic and renal vascular effects of administering a selective and a nonselective ET_A/ET_B receptor antagonist.[66] The selective ET_B receptor antagonist decreased RBF and increased renal vascular resistance, suggesting that ET may be maintaining a "tonic" renal vasodilation. The effects are consistent with the renal abundance of ET_B receptors. In contrast, inhibition of ET_A receptors has no effect on RBF. However, ET_A receptor–mediated vasoconstriction predominates during inhibition of NO synthesis in the kidneys. Acute systemic NO synthetase inhibition produces a marked increase in systemic arterial pressure and a renal vasoconstrictive response, which is due to elevation of both glomerular afferent and efferent vascular resistances.[67] This increase in intrarenal resistances, caused by inhibition of NO synthetase, was partially abolished by ET-1 blockade. However, simultaneous inhibition of ET-1 and Ang II restores RBF to control levels, showing that the vasoconstrictive effects of ET-1 and Ang II counterbalance the vasodilative effect of NO in the kidneys.

Renal Tubular Effects

As mentioned previously, ET_B is the major ET receptor expressed in renal tubules. Endothelin A receptors are present in different parts of the nephron, but their role in the regulation of fluid excretion is speculative. Endothelin 1, through ET_B receptors, affects the distinct parts of the nephron differently. The administration of big ET-1 results in diuresis and natriuresis.[68] It has been proposed that local conversion of big ET-1 from ET-1 by local ECE allows the active peptide to gain access to renal sites not accessible to

exogenous ET-1. In the proximal tubule, ET has a biphasic effect,[69] ie, low concentrations of ET-1 increase fluid transport, whereas high concentrations decrease fluid transport via PKC-, cyclooxygenase-, and lipoxygenase-dependent mechanisms. Therefore, increases in ET-1 elicit natriuretic and diuretic responses by the proximal tubule. In the thick ascending limb of the loop of Henle, ET inhibits chloride flux via a NO-dependent mechanism.[70] In the cortical collecting duct, ET-1 inhibits Na^+ and water reabsorption and decreases mineralocorticoid and arginine vasopressin (AVP)–stimulated Na^+ and Cl^- reabsorption. The inner medullary collecting ducts (IMCDs) not only synthesize the highest amount of ET-1 but also are the major site of receptor expression, suggesting they play a more important role than other nephron segments. In the IMCDs, the ET_B receptor stimulates NO and cyclic guanosine monophosphate production, reduces the activity of Na^+/K^+ adenosine triphosphatase by stimulation of prostaglandin E_2 synthesis, and thus reduces Na^+ transport. Endothelin 1 also inhibits AVP-induced changes in water permeability by decreasing cyclic adenosine monophosphate accumulation.[71,72] The role of endogenous ET on tubular function has been studied by using ET receptor antagonists. Pretreatment of rats with A-192621.1, a highly selective ET_B antagonist, significantly reduces the diuretic and natriuretic responses induced by big ET-1.[73] This finding is consistent with the high abundance of ET_B receptors in IMCD epithelium, the main inhibitory site of ET-1 on Na^+ and water reabsorption. As shown by Abassi et al,[74] activation of ET_B receptors increases the release of NO. When NO production is inhibited during the stimulation of ET_B receptors, the diuretic and natriuretic responses to big ET-1 are blunted. Furthermore, ET-1 acts through ET_B receptors to induce transient medullary vasodilation, which may contribute to the diuretic/natriuretic effects of locally produced ET-1 in the renal medulla. The ET-1 urinary levels reflect only the peptide produced in the kidneys and are derived almost entirely from renal tubular secretion. Most of the circulating ET-1 filtered by the glomerulus is degraded by NEP, which accounts for the exogenously labeled ET-1 that enters the circulation but is not excreted.

INTEGRATIVE ROLE OF ET IN SALT METABOLISM AND HYPERTENSION

The hemodynamic and renal effects of ET that have been examined in animals[75] and humans[76] suggest that the major role of this hormone is to supplement the natriuretic effect produced by the withdrawal of Ang II during high sodium intake or volume expansion. As shown in Figure 4, excessive sodium intake is followed by extracellular fluid volume expansion, which leads to an increase in venous return, end-diastolic pressure, and cardiac output. This is accompanied by suppression of sympathetic activity, which induces renal vasodilation and elevation of renal interstitial pressure.[32] These changes are followed by a decrease in the release of renin and accordingly in the circulating amount of this hormone and in the concentration of angiotensin contained in the kidneys.[32] These changes, along with a decrease in angiotensin antinatriuretic effect, were considered the causes of the natriuretic response responsible for maintaining the sodium balance.[32] However, it could be argued that the total withdrawal of important pressor and vasoconstrictive factors such as the renin-angiotensin system, vasopressin, and norepinephrine may notably compromise blood pressure levels necessary to maintain pressure natriuresis and/or may lead to inadequate sodium excretion. These deficiencies should be compensated by the activation of the systemic vasoconstrictive effect of ET (exerted through ET_A receptors) and the simultaneous renal vasodilative and natriuretic effects of ET produced by ET_B receptors. Pollock and Pollock[77] supported this assumption. They observed that high sodium intake in normal rats increased the basal levels of mean arterial pressure (MAP) by 12 mm Hg. The specific blockade of ET_B receptor in rats fed a high-sodium diet was followed by a rapid (few hours later) increase in MAP of 55 mm Hg (from 115±2 to 170±3 mm Hg). In contrast, the elevation of MAP in animals fed a low-sodium diet and treated with ET_B receptor blockers was not significant. Furthermore, it was shown that the hypertensive effect in animals fed a high-sodium diet produced by the blockade of ET_B receptors was due to indirect activation of ET_A receptors because their blockade reduced blood pressure to a normal level of 113±3 mm Hg.

Gariepy et al[78] further supported the idea that ET plays an important role in facilitating sodium excretion through activation of ET_B receptors. These investigators overcame the problems of congenital distal intestinal aganglionosis in rats with genetic knock-out ET_B receptor with the administration of dopamine hydrolase. These animals developed severe hypertension at 168±7 mm Hg, which contrasted with the small increase at 121±2 mm Hg seen in wild-type rats. No significant differences were observed between the 2 groups when they were fed a normal-sodium diet. Knock-out animals exhibited a MAP of 107±2 mm Hg, whereas wild-type animals had a MAP of 110±3 mm Hg.

Another situation in which the effects of ET may be effectively manifested is when the release of renin and the levels of circulating Ang II cannot be adjusted to changes in extracellular fluid volume expansion or sodium intake. This effect could be produced by alterations in the mechanism that controls renin exocytosis such as decreased pulse pressure (atherosclerosis) or cellular entry of calcium. This situation can be mimicked in experimental animals by the

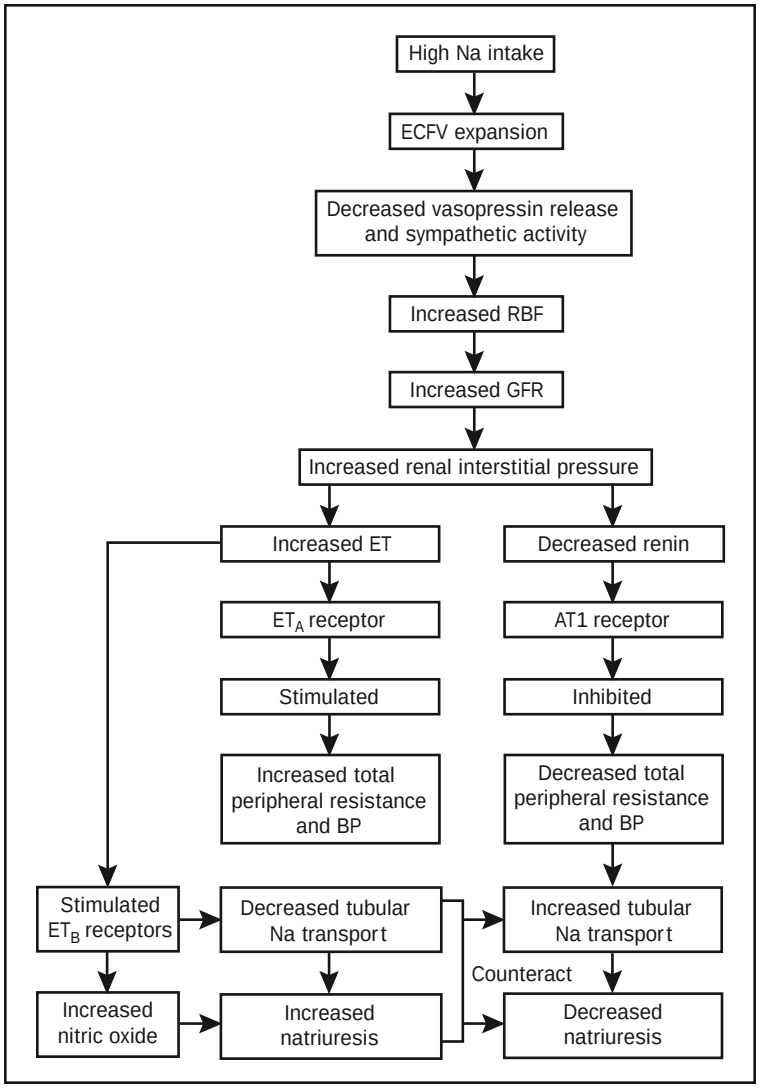

FIGURE 4. Reciprocal changes in the endothelin (ET) and renin-angiotensin systems during high sodium (Na) intake. The ET minimizes a decrease in blood pressure (BP) due to withdrawal of renin and other vasoconstrictive systems and facilitates natriuresis by inhibiting Na transport (reabsorption) in all tubular segments. Inhibition of Na transport is produced by ET_B receptor stimulation and increased nitric oxide (NO) synthesis. Endothelin also is effective in preserving Na balance during periods of inappropriately high levels of renin. AT1 = angiotensin I; ECFV = extracellular fluid volume expansion; GFR = glomerular filtration rate; RBF = renal blood flow.

so-called slow pressor responses to Ang II, which consists of administering subpressor doses of Ang II that produce small but prolonged sodium retention and a delayed (3-10 days) chronic elevation of blood pressure.[79] Under these conditions, administration of a specific ET_A receptor antagonist (EBT 627) reduced MAP to normal levels without altering the blood pressure of normotensive controls given a high-salt diet and no infusion of angiotensin.[79] Furthermore, in this angiotensin-induced model of hypertension, specific blockade of ET_B receptors produced a further 18% increase in MAP over the hypertensive levels already induced by Ang II infusion.[79]

According to these findings, ET-dependent hypertension should be induced when plasma levels of renin and/or angiotensin are inappropriately high with respect to the amount of sodium intake or fluid volumes, in which case inactivation of ET facilitates sodium excretion through ET_B receptors. This is accomplished by alterations of ET_B receptors, which produce a deficient modulation of renal response to the high-sodium diet.

TABLE 2. **Endothelin Expression in Models of Experimental Hypertension***

Hypertension model	Vascular ET-1 mRNA	Participation of ET-1	ET-1 mRNA	Effects of ET blockade
Dahl salt-sensitive rat	High	Yes	High	Decreased blood pressure
DOCA-salt hypertensive rat	Not known	Yes	No change	Slightly decreased blood pressure
DOCA-salt–treated SHR	High	Yes	High	Decreased blood pressure
SHR	Normal/low	No	No change	No effect
SHR stroke-prone	Not known	Yes	No change	Decreased blood pressure
SHR + L-NAME	Normal	No	No change	No effect
L-NAME-induced hypertension	Normal	No	No change	No effect
Angiotensin II–infused hypertension	Not known	Yes	No change	Decreased blood pressure
2K-1C Goldblatt hypertension	Normal	No	No change	No effect
1K-1C Goldblatt hypertension	Normal	Not known	No change	No effect

*DOCA = desoxycorticosterone; ET-1 = endothelin 1; 1K-1C = 1-kidney 1-clip; 2K-1C = 2-kidney 1-clip; L-NAME = N^G-nitro-L-arginine methyl ester; mRNA = messenger RNA; Nx = uninephrectomy; SHR = spontaneously hypertensive rat.

POSSIBLE ROLE OF ET IN HYPERTENSION

The ET system has been implicated in the pathogenesis of hypertension on the basis of studies that showed that infusion of ET-1 increased blood pressure in animals[80] and humans[75] and that blocking of the ET system decreased blood pressure. We review the role of ET in diverse experimental models of hypertension and then review human studies of the effect of ET on hypertension.

ROLE OF ET IN EXPERIMENTAL MODELS OF HYPERTENSION

The role of ET in experimental models of hypertension is not fully understood. Significant increases in plasma ET-1 levels are seen consistently only in certain models of accelerated/malignant hypertension, such as caffeine-treated renovascular hypertensive rats[81] and desoxycorticosterone acetate (DOCA)-salt–treated spontaneously hypertensive rats (SHRs).[81,82] Also, expression of ET or activation of the ET system is seen only in a few experimental models of hypertension.[82] However, several other models respond to ET blockade, suggesting that the ET system is implicated in hypertension, despite the lack of clear activation of the ET system. The activity and/or expression of the ET system in distinct models of experimental hypertension is depicted in Table 2.

Dahl Salt-Sensitive Hypertension. Growing evidence shows that the ET system is important in the initiation and/or maintenance of hypertension in several models of salt-sensitive hypertension. For instance, Dahl salt-sensitive (DS) rats on a normal-salt diet have increased renal ET-1 expression compared with Dahl salt-resistant rats, and expression is increased further in response to a high-salt diet.[76,83] Interestingly, renal expression of ET-1 correlates with systolic blood pressure in DS rats. These animals also had a notable shift in the pressure-natriuresis relationship, indicating impaired capability to excrete Na^+ and water. This dysfunction exists in prehypertensive DS rats and worsens progressively with high-salt intake. Dahl salt-sensitive rats not only exhibit an altered expression of ET-1 but also have an exaggerated vascular reactivity in response to ET-1 compared with Dahl salt-resistant rats, regardless of salt intake. Together, these studies suggest that the ET system plays an important role in maintaining hypertension in DS rats. However, the increased activity of ET may be a response to induce sodium excretion, produced by inappropriate sodium retention (Figure 4).

DOCA-Salt Hypertensive Models. The ET system is implicated in the pathogenesis of the hypertension observed in DOCA-salt hypertensive rats[81,82,84-86] and the DOCA-salt–treated SHRs.[81,82] Both models have increased levels of ET-1 mRNA in the vessel walls. Also, the DOCA-salt hypertensive rats reportedly have increased ET_B expression,[84,85] which may be due to reduced ET-1 levels in the renal medulla (despite increased vascular ET expression). This decrease in medullary ET-1 may enhance water and salt reabsorption in the collecting duct and thus contribute to the development of hypertension. In fact, by further impairing the activation of the ET_B receptor, a more severe hypertension develops, as observed in ET_B receptor–deficient DOCA-salt hypertensive rats.[85] Finally, further evidence suggesting an important role for medullary ET in the DOCA-salt model of hypertension comes from studies that show an apparent interaction between ET-1 and vasopressin. Administering a vasopressin type 1 receptor antagonist decreased blood pressure and vascular expression of ET-1 and prevented vascular hypertrophy.[87] The importance of this interaction is supported further by the finding that DOCA-salt does not produce hypertension in Brattleboro rats, which are vasopressin deficient.[88]

SHR Hypertensive Model. Most studies suggest that the ET system does not play a prominent role in the SHR model of hypertension. However, it may be an exacerbating factor in some SHR strains when they are exposed to additional factors that render the animals sensitive to salt. For instance, DOCA-salt–treated SHRs have increased

vascular ET-1 expression. The ET system also may play a role in stroke-prone SHRs rendered salt sensitive via unilateral nephrectomy[89,90]; however, this appears to be through a distinct mechanism. These rats have a greater increase in ET_A than in ET_B receptor density in the kidneys in response to a high-salt diet than their SHR counterparts.[90] This increase in the ET_A/ET_B receptor ratio, together with decreased affinity of the ET_B receptor, leads to an extensive predominance of ET_A receptor–mediated effects. Thus, SHRs rendered salt sensitive via unilateral nephrectomy that are on a high-sodium diet have unopposed activation of ET_A receptors, with consequent augmentation of renal vasoconstriction and stimulation of sodium reabsorption (via stimulation of the epithelium sodium channel in the distal nephron) that leads to Na^+ retention and hypertension.

Subpressor Ang II–Induced Hypertension. Chronic infusion of a subpressor dose of Ang II into rats causes salt-sensitive hypertension[91] with significant shifting of the pressure-natriuresis curve. The ET system is believed to play a prominent role in the development of hypertension in this model. Indeed, the hypertensive response to a subpressor dose of Ang II is accompanied by an increase in oxidative stress with a subsequent increase in F_2-isoprostanes and in the expression and intrarenal levels of ET-1.[28,29,91] The increased ET-1 level in this model is believed to result from an increased level of F_2-isoprostane, which can stimulate ET-1 synthesis in endothelial cells.[92] Furthermore, treating the hypertensive animals with an antioxidant prevented an increase in blood pressure, F_2-isoprostane level, and ET-1 level.[29] Furthermore, treating the animals with either an ET_{A-B} or an ET_A receptor antagonist prevented the development of hypertension.[28] The antihypertensive effect of the ET_A receptor blocker was greater in rats fed a high-salt diet.[79] Together, these results provide compelling evidence that ET is implicated in this form of hypertension. In addition, as in the previously discussed models of experimental hypertension, salt intake appears to be an important determinant in ET-mediated hypertensive responses. Additional evidence for this modulatory effect of salt intake on ET stems from the following observation. First, Ang II induces ET-1 release from endothelial cells and enhances the vascular reactivity to ET in animals on a normal-sodium diet, but both of these effects are amplified notably if the animals have been maintained on a high-salt diet.[93] Second, chronic Ang II infusion, combined with a high-salt diet, increased the renal cortical and outer medullary immunoreactive ET-1 content. This increase in cortical ET-1 level may contribute to hypertension and to a decline in renal function, whereas an increased ET-1 level in the outer medulla may enhance medullary blood flow and Na^+ excretion. In contrast, a high-salt diet, with or without Ang II infusion, reduced inner medullary immunoreactive ET-1 content. It is unclear whether this surprising decrease is due to decreased ET-1 synthesis or enhanced release of ET-1 from tissue stores in response to salt loading as a means of reducing Na^+ reabsorption. However, this exemplifies the compartmentalization and complexity of the renal ET system in the pathogenesis of hypertension during Ang II infusion.

Role of ET-1 in Experimental Renovascular Hypertension. The role that ET-1 plays in 2-kidney 1-clip Goldblatt hypertension (2K-1C GH) is unclear. Infusing Ang II increases oxidative stress and plasma F_2-isoprostane levels,[91] which in turn can induce the release of ET-1 from endothelial cells and VSMCs.[94] Therefore, ET-1 presumably may be formed by endogenous Ang II (2K-1C GH rat),[81] thus raising the possibility that ET plays a role in renovascular hypertension. However, ET antagonism has not been found to consistently decrease blood pressure and expression of the ET system, although ET-1 mRNA in the blood vessel wall may be slightly elevated.[81,95,96] These results suggest that ET is not implicated in this model of hypertension. However, in low-renin, volume-dependent, 1-kidney 1-clip Goldblatt hypertensive rats, the ET system appears to be activated, as it is in caffeine-treated 2K-1C GH rats.[81] Thus, ET again becomes a key factor when an experimental model is rendered salt sensitive or in cases of accelerated hypertension. Thus, further studies are needed to elucidate the role of the ET system in renovascular hypertension.

To summarize, the one constant that is consistently present in all ET-dependent models of experimental hypertension is their salt-sensitive nature. Although ET may be an important facilitator for sodium excretion in normal animals, alterations in this system may contribute to the impaired capacity to excrete salt and thus to the development of salt-sensitive hypertension (Figure 4). Furthermore, because a high-salt diet itself can stimulate the production of oxidative stress,[91,97] ET-1,[98] and ultimately cardiac hypertrophy,[99] it is tempting to speculate that abnormalities in the ET system may play a role in the particularly aggressive vascular and renal disease that characterizes many salt-sensitive hypertensive models.

Role of ET in Human Hypertension

The role of ET in human hypertension is poorly understood. Human trials with ET receptor antagonists have not shown the promising effects observed in animal disease models.[8] However, these trials primarily studied "generic" essential hypertension rather than a patient population that may be more susceptible to ET blockade (ie, salt-sensitive forms of hypertension). Indeed, in addition to the abundant data available about the role of ET in salt-sensitive experimental hypertension, there are provocative human data that need to be investigated further.

Plasma concentrations of ET-1 reportedly are increased in humans with salt-sensitive essential hypertension compared with healthy persons.[86,100-102] This does not appear to be due solely to salt retention because the plasma levels of ET-1 increased by a greater magnitude in salt-sensitive patients than in salt-resistant patients with salt loading. However, not all studies have found increased plasma ET-1 levels in these patients.[103] In addition, many confounding factors can blur the apparent relationship between ET-1 and salt sensitivity. For instance, increased plasma ET-1 levels or enhanced endothelial expression of the *ET-1* gene[104] are found in some patients with moderate to severe essential hypertension[105,106] and in patients with pheochromocytoma.[107] Endothelial cell damage also may cause an increase in plasma ET-1. In fact, long-term high-salt intake impairs endothelial function, which may contribute to the exaggerated blood pressure response of these subjects to salt loading.[108] Finally, increased plasma ET-1 levels are associated with patients with diverse renal diseases or end-organ damage.[102,109,110] Despite these caveats, the antihypertensive effects of ET_A/ET_B receptor antagonists in salt-sensitive essential hypertension suggest that *high circulating levels of ET-1* may contribute to the increased peripheral resistance observed in these forms of hypertension.[111]

Conversely, *decreased renal medullary ET levels* also may contribute to the development of salt-sensitive hypertension in humans. This is because ET in the renal medulla facilitates salt excretion. Thus, a deficiency in the ET system at this level leads to an impaired capacity to excrete salt. Of note, a negative correlation exists between *urinary* levels of ET-1 and MAP in normotensive and hypertensive subjects; thus, urinary ET-1 levels usually are decreased in essential hypertension.[112] However, the lowest levels are observed in salt-sensitive hypertensive patients.[112] Indeed, during salt load, salt-sensitive patients have a blunted renal ET-1 response: urinary ET levels increase less than in salt-resistant patients. Therefore, it is tempting to speculate that this decreased urinary ET-1 level may be responsible for the decreased natriuresis in salt-sensitive patients. Thus, the ET system could participate in salt-sensitive hypertension with either an exaggerated vasoconstrictor contribution or diminishing natriuresis.

Finally, we present some thoughts regarding the initial trials that evaluated the efficacy of ET antagonist in essential hypertension. These trials strongly suggest that most essential hypertension is only moderately susceptible to therapy with ET blockade. However, several issues remain unresolved and require further investigation. First, previous trials used nonselective ET_{A+B} antagonist. The selective ET_A antagonist has a theoretical benefit in that it blocks the hypertensive properties of the ET_A receptor while preserving the natriuretic properties of the ET_B receptor. Thus, it is important to determine whether selective ET_A receptor blockade is more beneficial in this setting. Second, because the ET system seems to be important, mainly in the setting of salt-sensitive and accelerated hypertension, ET blockers may be more effective in these forms of hypertension than in non–salt-sensitive mild essential hypertension. Third, ET antagonists were used as monotherapy. Because ET has important interactions with other systems (ie, Ang II, AVP) and ET antagonists have the potential for improving salt handling, it should be determined whether ET antagonists facilitate the actions of other antihypertensives (or have a synergistic effect). Finally, the only parameter that was examined thoroughly in previous trials was blood pressure control. However, because of the importance of the ET system on tissue/organ damage, ET antagonists may provide additional protection against end-organ damage, independent from blood pressure control. Trials that address these issues are needed to determine whether manipulation of the ET system is of benefit in treating certain forms of hypertension and thus avoiding the aggressive progression of end-organ damage that frequently accompanies hypertension.

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