Published in final edited form as:
Curr Mol Pharmacol. 2011 November 1; 4(3): 176–186.

Modulators of the Vascular Endothelin Receptor in Blood Pressure Regulation and Hypertension

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Abstract

Endothelin (ET) is one of the most investigated molecules in vascular biology. Since its discovery two decades ago, several ET isoforms, receptors, signaling pathways, agonists and antagonists have been identified. ET functions as a potent endothelium-derived vasoconstrictor, but could also play a role in vascular relaxation. In endothelial cells, preproET and big ET are cleaved by ET converting enzymes into ET-1, −2, −3 and −4. These ET isoforms bind with different affinities to ET_A and ET_B receptors in vascular smooth muscle (VSM), and in turn increase $[Ca^{2+}]_i$, protein kinase C and mitogen-activated protein kinase and other signaling pathways of VSM contraction and cell proliferation. ET also binds to endothelial ET_B receptors and stimulates the release of nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor. ET, via endothelial ET_B receptor, could also promote ET re-uptake and clearance. While the effects of ET on vascular reactivity and growth have been thoroughly examined, its role in the regulation of blood pressure and the pathogenesis of hypertension is not clearly established. Elevated plasma and vascular tissue levels of ET have been identified in salt-sensitive hypertension and in moderate to severe hypertension, and ET receptor antagonists have been shown to reduce blood pressure to variable extents in these forms of hypertension. The development of new pharmacological and genetic tools could lead to more effective and specific modulators of the vascular ET system for treatment of hypertension and related cardiovascular disease.

Keywords

endothelium; nitric oxide; smooth muscle; calcium; blood pressure

INTRODUCTION

The endothelium is an integral component of the vascular wall and a major regulator of vascular reactivity and blood pressure. The endothelium releases vasodilator factors such as nitric oxide (NO), prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factors (EDHFs) [1–4]. A decrease in the release of endothelium-derived vasodilators reduces vascular relaxation and consequently increases vascular resistance and blood pressure and leads to hypertension (HTN). The endothelium also releases vasoconstrictor factors such as thromboxane A2, angiotensin II (AngII) and endothelin (ET), one of the most potent vasoconstrictors described [5]. Since its discovery in 1988, several ET isoforms and ET receptor subtypes have been identified in the kidney and blood vessels and have been shown to affect sodium balance and vascular function [5–8]. While the prominent effects of ET on renal sodium absorption and vasoconstriction are expected to increase blood pressure, the

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role of ET and its receptors in the pathogenesis of HTN has not been clearly defined. Importantly, the plasma and vascular tissue levels of ET are elevated in some but not all forms of experimental and human HTN [9,10]. ET receptor antagonists may decrease blood pressure in experimental HTN [11]. Also some studies have demonstrated significant antihypertensive activity of ET antagonists such as bosentan in essential HTN [12], highlighting the importance of studying the effects of modulators of the ET system in the regulation of blood pressure and in HTN.

This review will discuss reports published in the PubMed database to provide insight into the role of ET and vascular ET receptors in the regulation of vascular function and blood pressure, and how alterations in their expression and signaling mechanisms could lead to HTN. The review will first describe the various ET isoforms, their plasma and vascular tissue levels and the various pathways involved in their synthesis and metabolism. The ET receptor subtypes, their vascular tissue distribution and signaling pathways will then be described. We will finally discuss the pathophysiological changes in ET metabolism and function in specific forms of experimental HTN and the potential use of ET receptor antagonists in the management of human HTN.

ET Synthesis, Metabolism and Clearance

The ET peptide family includes the 21-amino acid peptides ET-1, −2, −3, and −4, and several 31-amino acid forms. ET-1 is mainly released from the endothelium and acts in an autocrine or paracrine fashion on ET receptors in endothelial cells and vascular smooth muscle (VSM) to stimulate vascular contraction and cell growth [13]. ET-1 is also produced by airway epithelial cells, cardiomyocytes, fibroblasts, neurons and macrophages [14]. ET-2 is produced by intestinal epithelial cells. ET-3 is produced by intestinal epithelial cells, renal tubular epithelial cells and neurons. ET-4 is produced by the intestinal, lung and renal epithelial cells [15,16].

In endothelial cells, ET synthesis is initiated from preproET gene transcription (Fig. 1), which can be regulated by c-fos, c-jun, nuclear factor-1, AP-1 and GATA-2 [17]. The translation of preproET mRNA produces long 203-amino acid proteins called preproETs. PreproETs are then cleaved by furin-like protease to biologically inactive 37- to 41-amino acid big ETs. Big ET-1 and big ET-2 are cleaved at Trp_{21}-Val_{22} by ET converting enzyme-1 (ECE-1) and ECE-2 to produce ET-$1_{1–21}$ and ET-$2_{1–21}$, respectively. Mast cell chymases could also cleave big ET-1 and big ET-2 at Tyr_{31}-Gly_{32} to produce ET-$1_{1–31}$ and ET-$2_{1–31}$, respectively. Big ET-3 is cleaved at Trp_{21}-lle_{22} by ECE-1, −2 and −3 to produce ET-$3_{1–21}$, and at Gly_{31}-Leu_{32} by chymases to form ET-$3_{1–31}$ [13].

ECEs are members of the metalloprotease family. ECE-1 is produced by many cells including endothelial cells, has peak activity at neutral pH, and is processed both intracellularly and on the cell surface. ECE-1 expression is regulated by protein kinase C (PKC), ET_B receptor, ets-1 transcription factor, and cytokines. The specificity of ECE-1 may provide a target to alter ET-1 production in certain cardiovascular disease such as HTN [18]. ECE-2 is produced by several cells including neurons and has peak activity at pH 5.8, suggesting intracellular processing. ECE-1 and ECE-2 show preference for big ET-1 over big ET-2 and big ET-3. Interestingly, ECE-1 and ECE-2 deficient mice have high levels of mature ET peptides, suggesting that ECE-3 or another unidentified enzyme may carry out the final processing step of ET [13,19].

Regulation of the ET system takes place mainly at the gene transcription level. PreproET-1 mRNA is upregulated during cardiovascular stress and in response to vasopressors and vasoactive agents such as AngII, norepinephrine, vasopressin, and thrombin as well as inflammatory cytokines such as tumor necrosis factor-α, interleukins and transforming

growth factor-β [8,20,21]. PreproET-1 mRNA expression is upregulated by hypocapnia, and may be downregulated by hypoxia [22]. In endothelial cells, PreproET-1 mRNA expression initially increases then decreases by mechanical shear stress [23]. On the other hand, NO, PGI_2 and atrial natriuretic factor decrease PreproET-1 mRNA expression and ET-1 release from endothelial cells [24,25]. The synthesized ET-1 is stored in elongated Weibel-Palade bodies in endothelial cells. During endothelial cell activation, the Weibel-Palade bodies relocate from the cytoplasm towards the plasma membrane and release ET-1 by exocytosis [26].

The plasma and vascular tissue levels of ET-1 are very low. In healthy adults, plasma ET-1 levels range between 0.7 and 5 pg/mL [27]. In normal Wistar rats, plasma ET-1 levels range between 0.7 and 4.9 fmol/mL [28]. In isolated aorta of Sprague-Dawley rats, ET levels are 120 pg/g tissue [29]. The low plasma/vascular tissue levels of ET could be due to its continuous metabolism/clearance. In normal adults, the 24-hour urinary ET excretion show marked variability between 1.7 pg/mL and 6.8 ng/mL [30,31]. In the kidney, neutral endopeptidase restricts ET-1 turnover, and endopeptidase inhibitors increase urinary ET levels [32,33]. ET clearance may also be achieved by its reuptake by certain endothelial ET_B receptors (ET_BR) called “clearance receptors” (Fig. 1) [32].

ET Receptors

ET receptors include ET_AR and ET_BR. ET_AR and ET_BR are widely expressed in numerous tissues including the kidney and blood vessels [8,32,34,35]. ET_AR mediates vasoconstriction and VSM cell proliferation, whereas ET_BR is involved in endothelial cell survival, ET re-uptake and clearance, and release of NO and PGI_2 (Fig. 1), as well as the inhibition of ECE-1. Pharmacological studies suggest the presence of two ET_BR subtypes in endothelial cells and VSM; however, there is no molecular evidence to support this sub-classification. ET_AR and ET_BR are encoded by distinct genes located on chromosomes 4 and 13, respectively, and the receptors share 63% amino acid identity [9]. In Western blot analysis of vascular tissues ET_AR is detected as a prominent immunoreactive band at 59 kDa, and less dense bands at 44 and 32 kDa. ET_BR is often detected as two immunoreactive bands at 64 and 44 kDa.

ET_AR is expressed in VSM of most blood vessels (Table 1), and in airway smooth muscle, cardiomyocytes, liver stellate cells, hepatocytes, neurons, osteoblasts, melanocytes, keratinocytes, adipocytes and the reproductive system [8,20,32]. In VSM, ET_AR is upregulated by insulin and NO [36,37]. While ET_BR predominates in endothelial cells, it is also present in VSM of some vascular beds [38,39] (Table 1). ET_BR has been identified in the aorta, coronary arteries, mesenteric arteries, and veins of experimental animals and in human mammary arteries [40,41]. ET_BR is also present in the brainstem glia and neurons, atrial and ventricular myocardium and the atrioventricular conducting tissue [38,42]. ET_BR have also been localized in renal tubules and collecting duct epithelial cells, airway smooth muscle, liver hepatocytes, osteoblasts, central and peripheral neurons, multiple endocrine tissues and the reproductive tract [32,42]. In endothelial cells, ET_BR is upregulated by tumor necrosis factor-α and basic fibroblast growth factor [32]. Both ET_AR and ET_BR are widely distributed in blood vessels, the nervous systems, and the renal arterioles, glomerular capillaries and inner medullary collecting ducts [34,43]. Immunohistochemical analyses have identified ET_AR and ET_BR in the plasmalemma and cytosol of several cell types. ET_BR has also been identified in the nuclear envelope and nucleoplasm [44] (Table 1).

ET Receptor-Mediated Signaling

ET-1 administration in experimental animals *in vivo* is associated with a transient vasodilator and blood pressure depressor response [5], and these effects are likely due to

activation of endothelial ET_BR. In endothelial cells, ET_BR is coupled to activation of signaling pathways that increase the release of relaxing factors such as NO, PGI_2 and EDHF (Fig. 1). NO and NO donors may inhibit ET-1 release or counteract its renal effects and vasoconstrictor effects on VSM [6]. This may explain why *in vivo* administration of NOS inhibitors such as L-NAME could enhance ET-1 release from endothelial cells. ET_BR may also be involved in the release of other endothelium-derived vasodilators such as PGI_2 and EDHF [45–47] (Fig. 1).

In VSM, ET_AR is coupled to $G_{q/11}$ protein to activate phospholiphase C-β (PLC-β), resulting in the breakdown of phosphatidylinositol 4,5-bisphosphate and the generation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol [48] (Fig. 2). IP_3 stimulates Ca^{2+} release from the intracellular Ca^{2+} stores in the sarcoplasmic reticulum. ET also stimulates Ca^{2+} influx from the extracellular space through plasmalemmal Ca^{2+} channels [49,50]. The ET/ET_AR-induced increase in diacylglycerol stimulates PKC activity [51–53]. Other ET_AR-mediated signaling pathways include phospholipase D with generation of diacylglycerol, phospholipase A_2 with release of arachidonic acid, the Na^+/H^+ exchanger, Src-family tyrosine kinases, phosphatidylinositol 3-kinase (PI_3K), mitogen-activated protein kinase (MAPK), p38 MAPK, and c-Jun-NH2-terminal kinase (JNK) [9,20,32,54].

ET/ET Receptors-Mediated Responses

ET-mediated cellular responses are dependent on both the ET agonist and the ET receptor subtype. ET-1 activates ET_AR in VSM and produces strong vasoconstriction, as well as proinflammatory, fibrosis and mitogenic effects, which could play a role in vascular injury and remodeling [32] (Fig. 2). ET-1, −2 and −3 could also enhance vascular contraction by activating ET_BR in VSM [55]. Activation of both ET_AR and ET_BR in the renal vasculature could promote renal vasoconstriction and increase $[Ca^{2+}]_i$ signaling in preglomerular VSM cells [56]. Also, the use of selective ET receptor agonists and antagonists have suggested the presence of small number of the vasoconstrictor ET_BR in human subcutaneous and rat mesenteric and pulmonary arteries, and therefore ET_BR agonists could elicit vasoconstriction *in vivo* and ET_BR-mediated contraction in isolated blood vessels [57,58].

Recent studies suggest an equally important role for endothelial ET_BR in the regulation of vascular tone, sodium balance and blood pressure [5]. ET-1 may produce vasodilatation via activation of endothelial ET_BR and enhanced eNOS activity and NO release [6] (Fig. 1). Activation of endothelial ET_BR could also produce blood pressure depressor effects via the release of PGI_2 in rabbit systemic vessels [45] and enhance vascular relaxation by releasing EDHF in rat mesenteric artery [46]. The role of ET_BR in the regulation of blood pressure is supported by the observation that ET_BR heterozygous (+/−) mice are hypertensive, possibly due to unbalanced activation of ET_AR by endogenous ET-1 [59]. The vascular ET_BR may also promote favorable inhibitory effects on vascular remodeling after injury [60]. ET_BR are also abundant in tubular epithelium and the renal medulla and ET-1 induced activation of ET_BR may inhibit sodium and water reabsorption [6,34]. Also, big ET-1 increases medullary blood flow in rats on a high salt diet consistent with a potential ET_BR-mediated natriuretic response to high salt intake, and a role for ET in regulating sodium excretion through activation of ET_BR [61].

ET_BR may also function as “clearance receptors” (Fig. 1). Studies have shown that the ET_BR selective antagonist BQ788 inhibits accumulation of intravenously administered radiolabeled ET-1 in the lungs and kidneys possibly by slowing its clearance from the circulation [32]. ET_BR deficient rats demonstrate increased plasma ET-1 levels and ET_AR expression, but decreased ET-1/ET_AR-mediated contraction in mesenteric arteries. This is

likely due to desensitization and uncoupling of the ET_AR due to the high levels of circulating ET-1 [55].

Modulators of ET Receptors

ET Receptor Agonists

ET_AR and ET_BR have different ligand specificity (Table 2) as well as affinity. ET_AR has subnanomolar affinity for ET-1 and ET-2 and markedly lower affinity for ET-3. ET_BR has equal subnanomolar affinities for ET-1, −2 and −3 [32]. The chymase-derived peptide $ET\text{-}1_{(1–31)}$ is a relatively selective agonist of ET_AR [62].

ET_BR agonists such as sarafotoxin 6c (S6c) induce small contraction in resistance vessels isolated from human subcutaneous fat and rat mesentery. S6c–induced contraction remains is not affected by ET_AR antagonists such as BQ610, and is slightly enhanced in the presence of the NOS inhibitor L-NAME and in endothelium-denuded vessels. These findings support that the vasoconstrictor ET_BR may partly contribute to the ET-mediated constriction in resistance vessels of human subcutaneous fat and in rat mesentery. In contrast, in isolated mouse vessels treated with L-NAME, S6c induces a minimal <5% contraction, suggesting a little role of ET_BR in the vasoconstriction of mouse vessels [63]. ET-3 has high affinity for ET_BR.

ET Receptor Antagonists

Several ET receptor antagonists have been used to examine the role of ET receptor in the control of vascular function and other systems (Table 2). ABT627, BQ123, and BQ610 are more selective toward ET_AR [64]. BQ123 and BQ610 inhibit ET-1 induced contraction in the mouse abdominal aorta, rat mesenteric vessels, and human subcutaneous vessels [38,63]. ET_BR antagonists such as A192621 and BQ788 have also been developed (Table 2). We have found that chronic treatment of rats with the ET_BR antagonist A192621 is associated with substantial increase in blood pressure, enhanced aortic contraction, and reduced endothelium-dependent aortic relaxation and NO production. The vasoconstrictive effects of A192621 were greater in rats on a high salt diet compared to those on normal salt diet, suggesting that endothelial ET_BR could influence basal vascular tone particularly during high salt diet [65].

Role of ET in Hypertension

The prominent and long-acting vasoconstrictor effects of ET-1 have suggested that it may play a role in the regulation of blood pressure and the pathophysiology of HTN. Although increased plasma levels of ET-1 have been demonstrated in some hypertensive patients, most patients with HTN show normal or slightly increased ET-1 levels [38]. Among African-Americans, plasma ET levels are increased in hypertensive compared with normotensive controls. On the other hand, among individuals with similar severity of HTN plasma ET levels are not higher in African-Americans compared with Caucasians [57]. Upregulation of the ET system is more commonly observed in severe cases of HTN associated with coronary artery disease, heart failure, atherosclerosis and pulmonary HTN. For example, plasma levels of ET-1 (5.15 pg/mL) and big ET-1 (25.7 pg/mL) are markedly elevated in patients with heart failure compared with control subjects (0.75 pg/mL and 7.7 pg/mL, respectively) [31].

The discrepancy in the plasma ET levels among hypertensive patients may be related to the rapid ET-1 clearance from the bloodstream. Also, ET is mainly secreted in a polarized from the endothelial cells to the underling VSM, leading to minimal increases in circulating plasma ET. Measurement of ET levels in vascular tissues may provide a more reliable tool

to determine the role of ET in HTN. For example, ET-1 mRNA expression is increased in the endothelium of subcutaneous resistance arteries from patients with moderate to severe HTN [38]. ET-1 tissue expression is also increased in other forms of human HTN including salt-sensitive HTN, low renin HTN, and obesity and insulin resistance-related HTN [66].

It is important to note that the tissue levels of ET-1 also show variability in animal models of HTN. ET-1 levels are elevated in the aortic wall of deoxycorticosterone acetate (DOCA)-salt hypertensive rats (730 pg/g) compared to control rats (120 pg/g) [29]. Also, the tissue levels of ET are markedly higher than the plasma levels, supporting the contention that ET tissue levels could be a better indicator of changes in the vascular ET system in HTN. ET-1 levels are also increased in the vascular wall of other salt-sensitive models of HTN such as DOCA-salt-treated spontaneously hypertensive rats (SHR) and salt-loaded stroke-prone SHR, Dahl salt-sensitive rats, AngII-infused rats and 1-kidney 1-clip Goldblatt hypertensive rats. In contrast, ET-1 vascular tissue levels are not increased in SHR, 2-kidney 1-clip hypertensive rats or L-NAME-treated rats [67].

The levels of ET in urine also show marked changes in HTN. For example, the 24 hour urinary excretion of ET-1 is markedly greater in hypertensive patients with heart failure (17.0 ng/g urinary creatinine or UC) as compared to control subjects (1.7 ng/g UC) [31].

ET Receptor-Mediated Responses in HTN

Both ET_AR, which mediate vasoconstriction, and ET_BR, which mediate vasodilation, could play a role in the regulation of blood pressure. An increase in the amount/activity of ET_AR or a decrease in the amount/activity of ET_BR is expected to cause an increase in blood pressure and HTN. However, the relationship between ET_AR and ET_BR in HTN is rather complex.

The number of vascular ET receptors could vary in different forms of HTN, and in various tissues isolated from subjects with the same form of HTN. For example, ET_BR are upregulated in the kidneys of DOCA-salt hypertensive rats, consistent with a role for ET_BR in the renal regulation of blood pressure [68]. ET receptors could also be downregulated by ET when a large amount of ET is produced in the vasculature. For instance, in DOCA-salt hypertensive rats the ET receptor density is reduced in some vascular beds, possibly due to increased vascular production of ET and consequent downregulation of ET receptors [69].

The variability in the ET system in HTN may not only involve the plasma and vascular tissue levels of ET and the amount of vascular ET receptors, but could also involve the vascular response to ET. Studies have shown that ET-1 induced contraction is increased in the coronaries of rat hearts during ischemia/reperfusion [70], and in the pulmonary artery of rat models of pulmonary HTN [71,72]. The augmented vascular reactivity to ET-1 in some forms of experimental HTN may be related to increased intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in VSM [56,73]. In contrast, ET-induced contraction is not enhanced in the aorta of SHR. ET-induced contraction is even decreased in the mesenteric arteries of DOCA-salt hypertensive rats and in ET_BR deficient rats possibly due to reduced ET_AR density as a result of its downregulation by the increased vascular ET production or decreased ET_BR-mediated ET-1 clearance [55,69].

In milder forms of HTN, the VSM of resistance arteries are restructured around a smaller lumen without true hypertrophy, resulting in reduced circumference and amplification of pressor stimuli. An example of this structurally based amplification is the enhanced vasoconstriction in isolated microvessels of SHR and renovascular hypertensive rats [38]. In severe forms of HTN and in secondary HTN, hypertrophic remodeling of VSM occurs [66]. Large conduit artery such as the aorta may demonstrate thickened tunica media, increased

collagen deposition and decreased compliance, leading to increased systolic blood pressure and pulse pressure. Because of the ET growth-promoting properties, it could play a role in the hypertrophy of VSM observed in severe HTN and in DOCA-salt hypertensive rats [38].

The responsiveness of endothelial ET_BR may also change in HTN. ET_BR-mediated vasorelaxation is greater in SHR and DOCA-salt hypertensive rats than normotensive rats. Thus while ET_AR may play a role in the development of DOCA-salt–induced HTN, ET_BR may protect against vascular and renal injury [74]. However, ET may decrease the release of endothelium-derived relaxing factor and thereby further enhance vasoconstriction in blood vessels of SHR [38]

ET Receptor Antagonists and Management of HTN

Because the effects of ET on vasoconstriction and VSM growth are mediated primarily via ET_AR, it is often thought that treatment with ET_AR antagonists would decrease blood pressure. However, the effects of ET_AR antagonists on blood pressure vary in normotensive and hypertensive animals. In normotensive rats, chronic administration of ET_AR antagonist does not affect blood pressure, suggesting that ET may not be involved in the regulation of normal blood pressure [75]. Also, some studies have shown that chronic treatment with the ET_AR antagonist A127722 or BQ123 only slightly lowers blood pressure in SHR and DOCA-salt hypertensive rats, and has no effect in renovascular model of HTN, suggesting that ET plays little role in these forms of HTN [57,76]. Other studies have shown that acute administration of the selective ET_AR antagonist ABT-627 markedly decreases blood pressure in DOCA-salt rats, and chronic treatment with ABT-627 suppresses the development of HTN [74]. Also, chronic treatment with the nonselective ET_A/ET_B antagonist bosentan decreases the development of HTN and vascular remodeling in DOCA-salt rats to a degree similar to that observed with ABT-627, suggesting that ET_AR plays a major role in DOCA-salt-sensitive HTN [74]. The net benefits of ET receptor antagonists in HTN could depend in their effectiveness to suppress the vasoconstrictive and growth promoting effects of ET. For instance, in hypertensive rats overexpressing ET-1, selective ET_AR antagonists lower blood pressure slightly, but markedly attenuate vascular hypertrophy, particularly in resistance arteries [67]. Similarly, treatment of SHR and DOCA-salt hypertensive rats with the nonselective $ET_{A/B}$ receptor antagonist bosentan reduces the blood pressure, but abolishes the vascular hypertrophy and remodeling of resistance arteries beyond what could be explained by the blood pressure lowering effect [38].

Clinical trials in humans have shown that non-selective ET receptor antagonists such as bosentan and combined treatment with ET_AR/ET_BR antagonists significantly reduce blood pressure [12,66]. In a study involving mild cases of essential hypertensive patients, a 4-week trial of bosentan reduced blood pressure to the same extent as the angiotensin converting enzyme (ACE) inhibitor enalapril. Also, some studies have suggested that treatment with ET receptor antagonists may improve hemodynamics in hypertensive patients with chronic heart failure (CHF) [77,78]. ET-1 plasma levels are elevated in CHF, correlate with both hemodynamic severity and symptoms, and are strong independent predictors of mortality in CHF. Combined ET_AR/ET_BR antagonists and selective ET_AR antagonists have been evaluated in patients with CHF showing considerable hemodynamic improvements, suggesting that ET receptor antagonists may have the potential to improve hemodynamics, symptoms, and prognosis in patients with CHF [77,78]. Despite these potential benefits and favorable short term effects on hemodynamics as suggested by some clinical trials, ET receptor antagonists have not been adopted for clinical management of CHF. ET receptor antagonists may also offer promise in primary pulmonary HTN [67]. For example, bosentan and ambrisentan have been shown to be effective in increasing 6 minute walk distance

(6MWD) and exercise improvements and reducing symptoms in idiopathic pulmonary HTN [79,80]. Examination of both the short-term and long-term beneficial effects of ET antagonists in HTN is also needed. The ET antagonist blood pressure lowering effects that might be modest in magnitude in the short-term could have significant long-term consequences. In pulmonary HTN, for example, it has been reported that the magnitude of improvement with ambrisentan, an orally active highly selective ET_AR antagonist with >4000-fold higher selectivity over ET_BR, was relatively small in the short-term, but could be meaningful as one-year survival was improved [81]. Thus, targeting the ET system using ET receptor antagonists may not only decrease blood pressure in HTN, but could also prevent target organ damage and other cardiovascular complications associated with HTN such as heart failure, atherosclerosis, coronary heart disease, restenosis after angioplasty and primary pulmonary HTN [67].

ET and Other Control Mechanisms of Blood Pressure

Despite the well-recognized effects of ET on vasoconstriction and remodeling, its effects on blood pressure have not been consistent. Some studies have shown that ET infusion increases blood pressure in rats [82], while other studies have shown slight or no effect [49]. The difference in the results could be related to differences in the activity of the ET from different commercial sources and rapid metabolism of administered ET. Also, the ET_AR-mediated vasoconstriction could be counterbalanced by ET_BR-mediated increase in endothelium-derived vasodilators. The relative number of ET_AR and ET_BR could also vary in different vascular beds due to developmental differences in ET receptor expression. This is particularly evident in the pulmonary circulation. For example, studies in fetal sheep *in utero* have shown that ET_AR activation may have a small role in maintaining basal fetal pulmonary vascular tone, while ET_BR activation produces marked pulmonary vasodilation [83]. ET could also affect other vascular, neuronal and renal control mechanisms of blood pressure. Likewise, the pressor effects of ET could be influenced by other factors such as the endothelial NO pathway, oxidative stress, the sympathetic nervous system, dietary salt intake, and the renin-angiotensin system.

NO plays a major role in the regulation of vascular function, and inhibition of NO production causes elevation of blood pressure in experimental animals. Interestingly, in rats chronically treated with NOS inhibitors such as L-NAME, ET_AR antagonists attenuate the early but not the late stages of HTN [75]. It has been suggested that ET may play a role in the development of early vascular lesions associated with NOS inhibition, at least within the kidney, possibly due to increased AngII activity. However, the mechanisms of the HTN associated with chronic NOS inhibition may be more complex and could vary with the animal species studied and the NOS inhibitor utilized [63].

Vascular oxidative stress is increased in parallel with the elevation of plasma ET levels in DOCA-salt hypertensive rats. Also, treatment of DOCA-salt hypertensive rats with ET_AR antagonist is associated with reduction in oxidative stress and reactive oxygen species. Oxidative stress is known to affect numerous vascular signaling pathways and several responses in the cardiovascular system including endothelial cell function and VSM growth. Thus the cardiovascular changes observed in DOCA-salt HTN could be partly due to increases in oxidative stress as a result of increased ET-1 expression/actions in this model [84].

High-salt diet intensifies the increase in blood pressure in salt-dependent hypertensive subjects. Salt-sensitive HTN is often associated with low plasma renin activity and elevated plasma levels of ET and catecholamines, suggesting synergistic effects between the sympathetic system, sodium sensitivity and the ET system in the elevation of blood pressure

[60]. Decreased endothelial NO production may also contribute to the development of salt-dependent HTN [85]. High salt diet is also associated with increased ET production, which in turn stimulates both ET_AR-mediated vasoconstriction and ET_BR-mediated vasodilation. In one study, chronic ET_BR blockade was associated with increased blood pressure in rats on normal salt diet, and the HTN was markedly enhanced in rats on a high-salt diet [65]. These findings are consistent with a role for ET-1 in regulating blood pressure particularly during high dietary salt intake [68].

The renin-angiotensin system is a major control mechanism in the regulation of blood pressure. Some of the effects of AngII such as VSM growth are mediated by ET-1 and ET_AR. Also, the stimulatory effects of AngII on ET production are more apparent in the blood vessels of SHR than normotensive rats [38].

Conclusions and Future Directions

The vascular ET system is an important modulator of vascular tone, growth, and the vascular control mechanisms of blood pressure. Although some studies suggest that ET infusion may cause elevation of blood pressure in some experimental animals, ET infusion does not always increase blood pressure, and not all animal models of HTN have high ET levels. ET appears to play a role in the elevation of blood pressure and vascular growth in moderate-to-severe HTN, salt-sensitive HTN, and in African-American subjects.

HTN is usually treated with diuretics, β-adrenergic blockers, ACE inhibitors, AngII receptor antagonists and long-acting Ca^{2+} channel blockers. However, in HTN the protracted increases in blood pressure are associated with progressive endothelial cell damage and consequent increases in ET-1 expression in blood vessels and the heart. ET in turn causes further elevation of blood pressure and promotes the progression of vascular and cardiac damage. Blocking the ET system may provide another approach to lower the blood pressure and to suppress the vascular and cardiac damage associated with HTN, and thereby improve the prognosis in hypertensive subjects. Currently available ET receptor antagonists reduce blood pressure in some forms of experimental HTN (Table 2). The effects of ET receptor antagonists in blunting vascular growth and endothelial dysfunction and in reducing the cardiovascular complications of HTN such as heart failure and stroke need to be further examined in clinical trials [67]. Since the final step of the biosynthesis of ET is catalyzed by ECEs, inhibitors of these enzymes may represent alternative therapeutic agents in HTN [18]. Another alternative way to modulate the ET system is to take a genetic approach and knock-out a specific ET isoform, ECE isozyme or ET receptor subtype. While the genetic approach has shown promising results in modulating cardiovascular disease in experimental animals, it remains to be validated in humans [57].

Finally, although the vascular ET system may play an important role in the vascular control mechanisms of blood pressure and the pathogenesis of some forms of HTN, the role of ET on the neuronal, hormonal and renal control mechanisms of the blood pressure should not be minimized. Also, HTN is a complex multifactorial disease that could involve abnormalities in several systems including ET, and modulating the ET system alone may not cure HTN. A multidisciplinary approach targeting the vascular ET system and the other neuronal, hormonal and renal pressor mechanisms should prove most effective in the management of HTN and associated cardiovascular disorders.

List of abbreviations

AngII angiotensin II

$[Ca^{2+}]_i$	intracellular free Ca^{2+} concentration
DOCA	deoxycorticosterone acetate
ECE	endothelin converting enzyme
ET-1	endothelin-1
ET_AR	endothelin receptor A
ET_BR	endothelin receptor B
HTN	hypertension
MAPK	mitogen-activated protein kinase
MLC	myosin light chain
NO	nitric oxide
PGI_2	prostacyclin
PKC	protein kinase C
S6c	sarafotoxin 6c
SHR	spontaneously hypertensive rat
VSM	vascular smooth muscle

Acknowledgments

This work was supported by grants from National Heart, Lung, and Blood Institute (HL-65998, HL-70659, and HL-98724) and The Eunice Kennedy Shriver National Institute of Child Health and Human Development (HD-60702).

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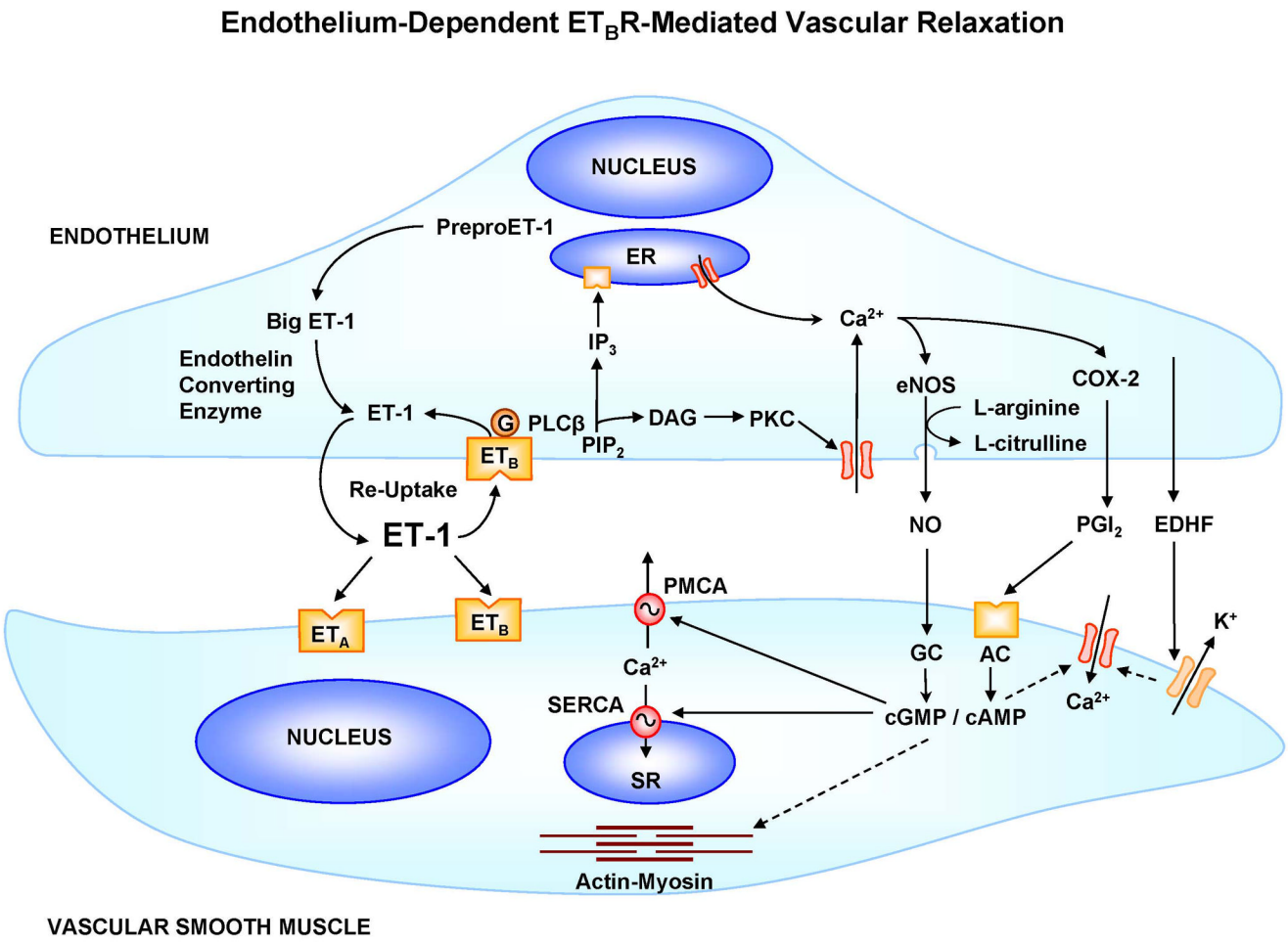

Fig. 1.

Endothelium-dependent ET_BR-mediated pathways of vascular relaxation. ET binds to ET_BR in endothelial cells to stimulate ET re-uptake and clearance. ET_BR also activates PLCβ and increases the hydrolysis of PIP_2 into IP_3 and diacylglycerol (DAG). IP_3 induces Ca^{2+} release from the endoplasmic reticulum (ER). The increased $[Ca^{2+}]_i$ activates eNOS, which in turn converts L-arginine to L-citrulline and increases NO production. NO diffuses into VSM, where it stimulates guanylate cyclase (GC) and increases cGMP production. cGMP decreases VSM $[Ca^{2+}]_i$ by decreasing Ca^{2+} entry via Ca^{2+} channels and increasing Ca^{2+} removal via plasmalemmal (PMCA) and sarcoplasmic reticulum Ca^{2+} ATPase (SERCA). cGMP also decreases the actin-myosin myofilament force sensitivity to Ca^{2+} and thereby causes vascular relaxation. Endothelial ET_BR is also coupled to stimulation of cyclooxygenases (COX-2) and increased prostacyclin (PGI_2) production. PGI_2 activates adenylate cyclase (AC) and increases the production of cAMP, which causes VSM relaxation by mechanisms similar to those of cGMP. Activation of endothelial ET_BR is also associated with increased release of EDHF, which activates K^+ channels and causes hyperpolarization and relaxation of VSM. Dashed arrows indicate inhibition.

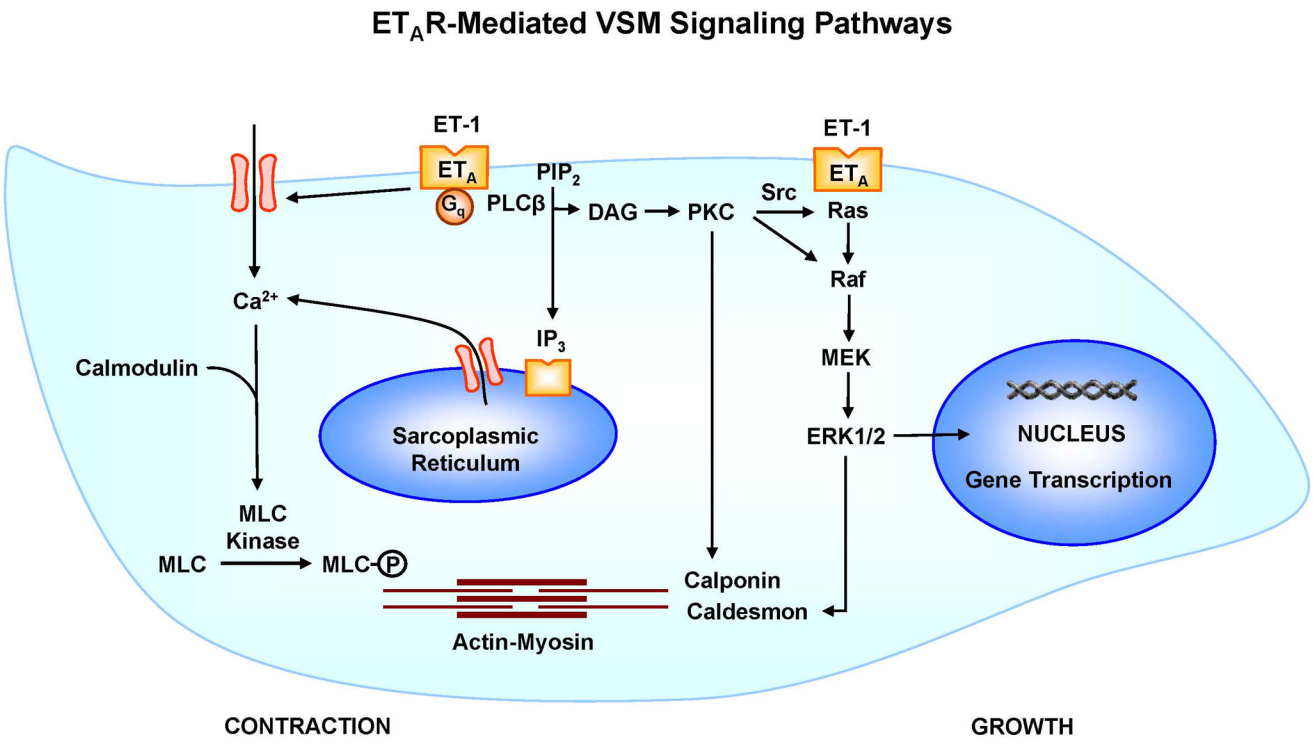

Fig. 2.

ET_AR-mediated pathways of VSM contraction and growth. The interaction of ET with ET_AR in VSM leads to activation of PLCβ and increased production of IP_3 and DAG. IP_3 stimulates Ca^{2+} release from the sarcoplasmic reticulum. ET also stimulates Ca^{2+} entry from the extracellular space through Ca^{2+} channels. Ca^{2+} binds calmodulin to form a complex, which causes activation of myosin light chain (MLC) kinase, increased MLC phosphorylation, actin-myosin interaction and VSM contraction. DAG activates PKC, which could phosphorylate the actin binding protein calponin or initiate a protein kinase cascade involving Raf, MAPK kinase (MEK) and MAPK (ERK1/2), leading to phosphorylation of the actin-binding protein caldesmon and thereby increases the myofilament force sensitivity to Ca^{2+}. ET_AR-mediated activation of MAPK could also induce gene transcription and VSM growth and proliferation.

Table 1

Examples of endothelin receptor distribution, function, and signaling pathways in the vascular system

	ET_AR	Endothelial ET_BR	VSM ET_BR
Molecular Weight	**Mainly 59 kDa**	**64/44 kDa**	**64/44 kDa**
Vascular Distribution			
Coronary Artery	+		+
Subcutaneous Arteries	+	+	+
Pulmonary Artery	+	+	+
Mammary Artery	+	+	+
Veins	+	+	+
Glomerular Capillaries	+	+	+
Cellular Distribution			
Endothelial Cells		+	
VSM	+		+
Sub-Cellular Distribution			
Cytosol / Plasmalemma	+	+	+
Nucleus		+	
Function	VSM Contraction Vasoconstriction VSM Growth Vascular Remodeling	VSM Relaxation Vasodilatation	VSM Contraction Vasoconstriction
Signaling Pathways	- Heterotrimeric G Protein - PLCβ, PLD - Increased $[Ca^{2+}]_i$ - PKC - MAPK	- G Protein - Increased $[Ca^{2+}]_i$ - NO - PGI_2 - EDHF	- G Protein - PLCβ, PLD - Increased $[Ca^{2+}]_i$ - PKC - MAPK

Table 2

Examples of ET Receptor Agonists and Antagonists and Their Selectivity to ET Receptor Subtypes

Compound	Chemistry	Receptor Specificity
Agonists		
ET_1		ET_A, ET_B
ET_2	Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe- Cys-His-Leu-Asp-Ile-Ile-Trp	ET_A, ET_B
ET_3		ET_B
IRL 1620	Suc-Asp-Glu-Glu-Ala-Val-Tyr-Phe-Ala-His-Leu-Asp-Ile-Ile-Trp	ET_B
S6c	Cys-Thr-Cys-Asn-Asp-Met-Thr-Asp-Glu-Glu-Cys-Leu-Asn-Phe- Cys-His-Gln-Asp-Val-Ile-Trp	ET_B
Antagonists		
A127722	2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(((dibutyl amino)carbonyl) methyl)pyrrolidine-3-carboxylic acid	ET_A
ABT627	(2R,3R,4S)-1-[(dibutyl carbamoyl)methyl]-2-(p-methoxyphenyl)- 4[3,4-(methylenedioxy)phenyl]-3-pyrrolidinecarboxylic acid	ET_A
Ambrisentan	(2S)-2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-methoxy- 3,3- diphenylpropanoic acid	ET_A
BMS182874	5-(dimethylamino)-N-(3,4-dimethyl-5-isoxazolyl)-1- naphthalenesulfonamide	ET_A
Bosentan	4-t-butyl-N-(6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2,2'- bipyrimidin-4-yl)benzenesulfonamide	ET_A, ET_B
BQ123	Cyclo(D-α-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl)	ET_A
BQ610	Homopiperidinyl-CO-Leu-D-Trp(CHO)-D-Trp-OH	ET_A
BQ788	N-cis-2,6-dimethylpiperidinocarbonyl-γ-methylleucyl- tryptophyl(COOMe)-norleucine	ET_B
LU135252	2-(4,6-dimethoxypyrimidin-2-yloxy)-3-methoxy-3,3- diphenylpropionic acid	ET_A
PD142893	Ac-D-(3,3-diphenylalanine)-leu-Asp-Ile-Ile-Trp-OH	ET_A, ET_B
PD145065	Ac-D-(5H–dibenzo[a,d]cycloheptene-5-glycine)-Leu-Asp-Ile-Ile- Trp-OH	ET_A, ET_B
PD147953 (FR139317)	N-(N-(N-((hexahydro-1H–azepin-1-yl)carbonyl)-L-leucyl)-1- methyl-D-tryptophyl)-3-(2-pyridinyl)-2-((1-(hexahydro-1H- azepinyl)carbonyl)amino-4-methyl-pentanoyl)-3-(-(1-methyl-1H-indolyl)propionyl)amino-3-(2-pyridyl)propionic acid	ET_A
PD156707	Sodium 2-benzo[1,3]dioxol-5-yl-4-(4-methoxy-phenyl)-4-oxo-3- (3,4,5-trimethoxy-benzyl)-but-2-enoate	ET_A
RES701-1	L-Tryptophan, glycyl-L-asparaginyl-L-tryptophyl-L-histidylglycyl- L-threonyl-L-alanyl-L-prolyl-L-α-aspartyl-L-tryptophyl-L- phenylalanyl-L-phenylalanyl-L-asparaginyl-L- tyrosyl-L-tyrosyl-, cyclic (9-1)-peptide	ET_B
SB209670	3-(2-(carboxymethoxy)-4-methoxyphenyl)-1-(3,4-(methylene dioxy)phenyl)-5-(prop-1-yloxy)indan-2-carboxylic acid	ET_A, ET_B
TAK044	cyclo(D-alpha-aspartyl-3-((4-phenylpiperazin-1-yl)carbonyl)-L- alanyl-L-alpha-aspartyl-D-2-(2-thienyl)glycyl-L-leucyl-D- tryptophyl) disodium salt	ET_A, ET_B
YM598	(E)-N-[6-methoxy-5-(2-methoxyphenoxy)[2,2'-bipyrimidin]-4-yl]- 2-phenylethenesulfonamide monopotassium salt	ET_A