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## Contrasting Actions of Endothelin ET<sub>A</sub> and ET<sub>B</sub> Receptors in Cardiovascular Disease

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### Abstract

First identified as a powerful vasoconstrictor, endothelin has an extremely diverse set of actions that influence homeostatic mechanisms throughout the body. Two receptor subtypes, ET<sub>A</sub> and ET<sub>B</sub>, which usually have opposing actions, mediate the actions of endothelin. ET<sub>A</sub> receptors function to promote vasoconstriction, growth, and inflammation while ET<sub>B</sub> receptors produce vasodilation, increases in sodium excretion, and inhibit growth and inflammation. Potent and selective receptor antagonists have been developed and have shown promising results in the treatment of cardiovascular diseases such as pulmonary arterial hypertension, acute and chronic heart failure, hypertension, renal failure and atherosclerosis. However, results are often contradictory and complicated because of the tissue-specific vasoconstrictor actions of ET<sub>B</sub> receptors and the fact that endothelin is an autocrine and paracrine factor whose activity is difficult to measure in vivo. Considerable questions remain regarding whether ET<sub>A</sub> selective or non-selective ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists would be useful in a range of clinical settings.

### Keywords

receptor localization; pulmonary hypertension; heart failure; chronic kidney disease

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Shortly after it was discovered that the vascular endothelium releases a peptide capable of profound vasoconstriction, a considerable amount of attention was paid to the potential actions of endothelin in the pathogenesis of cardiovascular disease. We have since learned a great deal about how this paracrine factor influences function in an extremely wide range of areas including neurotransmission, cell growth and epithelial transport, just to name a few. This myriad of activities has allowed the endothelin system to garner a tremendous amount of attention from the pharmaceutical industry with the development of numerous receptor specific and non-specific antagonists as well as efforts to identify drugs that inhibit endothelin synthesis. This work has led to new therapeutic approaches to pulmonary arterial hypertension and most likely other diseases in the not too distant future. In addition to this enormous drug discovery effort, it has also become clear that endothelin plays an important physiological role in maintaining blood pressure homeostasis, for example, by facilitating the excretion of a high salt diet. Because of the almost bewildering range of actions of the endothelin peptides, we limit this review to focus on the receptor-specific cardiovascular actions of endothelin.

### ENDOTHELIN PEPTIDES

Endothelin-1, a 21-amino-acid long peptide first isolated from the supernatant of cultured endothelial cells, is perhaps the most potent vasoconstrictor substance known (1). The human

endothelin family contains three 21-amino-acid long isopeptides, endothelin-1, endothelin-2 and endothelin-3 (ET-1, ET-2 and ET-3), which are each encoded by a separate, unique gene (2). The most extensively studied isopeptide, ET-1, is the major isopeptide of importance in the cardiovascular system (3,4). Endothelial cells are a major source of ET-1, making this peptide fairly ubiquitous, and constitutive release of the peptide from the endothelium may contribute to basal vascular tone. ET-1 is also produced by a variety of other cell types including the inner medullary collecting duct and most other nephron segments, neurons of the central nervous system, postganglionic sympathetic neurons, and monocytes/macrophages. Under pro-inflammatory conditions, vascular smooth muscle cells and pulmonary epithelial cells can also produce ET-1. ET-2 and its mouse or rat analog vasoactive intestinal contractor appear to be predominantly expressed in the intestine, colon, ovary and uterus, but expression has also been reported in brain and kidney. High concentrations of ET-3 have been measured in rat brain, pituitary, lung and intestinal homogenates. ET-3 is also produced by monocytes/macrophages and by renal tubular cells, although in much smaller quantities than ET-1. The human heart reportedly expresses all three endothelin isoforms.

The mature endothelin peptides are formed following a series of proteolytic cleavages of their approximately 200-amino-acid long precursor peptides (5). Pre-pro-endothelins are converted within the cell firstly to the inactive pro-endothelin peptide after removal of the signal peptide, and then into 38–41 amino acid long “big endothelins”, a step catalyzed by furin in the case of ET-1. The big endothelins then undergo final conversion to the active form of the peptide by endothelin-converting enzyme (ECE). The two major ECE isoforms are ECE-1 and ECE-2, which are membrane-bound zinc metalloproteases that show 59% amino acid sequence homology and cleave big ET-1 with much greater efficiency than either big ET-2 or big ET-3. In addition, a big ET-3-selective enzyme, ECE-3, has been purified from bovine iris microsomes. There are several sub-isoforms of both ECE-1 and ECE-2, with these sub-isoforms differing in sub-cellular localization, allowing the conversion of big endothelins to mature endothelins to take place both on the cell surface and intracellularly, allowing secretion of mature endothelins from endothelial and possibly other cells (reviewed by (6) and (5)).

## ENDOTHELIN RECEPTORS

The existence of multiple endothelin receptor subtypes was first hinted at by the characteristic biphasic blood pressure response to ET-1 in rats (1) and the differing pressor profiles of the three endothelin isoforms (2). In 1990, cloned cDNA sequences of two receptors for endothelin were published (7,8). When cells were transfected with the cloned cDNA, <sup>125</sup>I-labelled ET-1 was displaced from one receptor by all three peptides with ET-1 displaying the highest potency (7) whilst all three isopeptides displayed similar potencies in displacing <sup>125</sup>I-labelled ET-1 from the other receptor (8). These two receptors are respectively what are now known as the ET<sub>A</sub> and ET<sub>B</sub> receptors and are classified on the basis of their rank order of potencies for the endothelins, being ET-1 = ET-2 >> ET-3 for the ET<sub>A</sub> receptor and ET-1 = ET-2 = ET-3 for the ET<sub>B</sub> receptor (9). These are the two receptors that mediate the effects of the endothelins in mammals, although additional receptor subtypes have been identified in a small number of other species (10,11).

The amino acid sequences deduced from cloned ET<sub>A</sub> and ET<sub>B</sub> receptors predict that these receptors are heptahelical G-protein coupled receptors. In humans the ET<sub>A</sub> receptor is predicted to be 427 amino acids long, and shows approximately 64% sequence similarity to the predicted 442 amino acid long human ET<sub>B</sub> receptor (12). Endothelin receptors from several mammalian species display fairly high degrees of homology with the human ET<sub>A</sub> receptor (>90%) and ET<sub>B</sub> receptors (e.g. 97% for canine and 88% for rat) (9,13).

Endothelin receptors are expressed by a wide variety of cells and tissues. A complex array of signaling molecules are employed by the receptors to achieve the diverse effects of endothelins on their target cells. Shraga-Levine and Sokolovsky (14) demonstrated using fibroblasts over expressing either one or the other of the two receptor subtypes, that ET<sub>A</sub> and ET<sub>B</sub> receptors couple to multiple classes of G proteins, and that this coupling varies depending upon both the receptor subtype and the ligand bound to the receptor in question. In the coming years, it is undoubtedly expected that further research in this complex but important area will gradually delineate the pathways that underlie the many physiological and pathophysiological actions of endothelins on different cells types.

### **ET<sub>A</sub> and ET<sub>B</sub> receptors in the vasculature**

Both ET<sub>A</sub> and ET<sub>B</sub> receptors located on vascular smooth muscle mediate the potent vasoconstrictor effects that are characteristic of the endothelins (3). Although the precise signaling events responsible for endothelin-induced vasoconstriction in different vascular beds are still being actively investigated, it is commonly accepted that phospholipase C activation, inositol triphosphate generation and calcium mobilization from intra- and extra-cellular sources are involved. The ET<sub>B</sub> receptor exerts a dual role on vascular tone, as activation of ET<sub>B</sub> receptors located on the endothelium stimulates the production of nitric oxide and vasodilator cyclooxygenase metabolites, which exert vasorelaxant effects on the underlying smooth muscle (4). The predominant influence of endogenous endothelins on vascular tone and basal blood pressure is somewhat contentious. Acute ET<sub>A</sub> receptor blockade produces either a small decrease or no change in mean arterial pressure (15), perhaps suggesting that ET<sub>A</sub> receptors play relatively little role in determining basal vascular tone in healthy individuals. In contrast, acute and chronic ET<sub>B</sub> receptor blockade has consistently been shown to increase mean arterial pressure (16,17), an effect that appears to involve enhanced ET<sub>A</sub> receptor activation but is also compounded by the loss of ET<sub>B</sub> receptor-mediated NO production. Thus it could be argued that the ET<sub>B</sub> receptor plays a more important role in the control of basal blood pressure and vascular tone than the ET<sub>A</sub> receptor, by protecting the vasculature against the potent vasoconstrictor effects of endogenous endothelins. There is also evidence that endogenously produced endothelins in healthy humans contribute to vascular tone with a limited degree of vasoconstriction. Supporting this contention includes the demonstration of increases in forearm blood flow in response to local infusion of the ECE inhibitor phosphoramidon (18) or non-selective endothelin receptor blockade (19). Further, systemic administration of combined ET<sub>A</sub> and ET<sub>B</sub> antagonists has been shown to produce mild decreases in total peripheral resistance and mean arterial pressure in healthy humans (20).

In addition to actions on vascular tone, endothelins also promote growth and proliferation of vascular smooth muscle cells, an effect that appears to be ET<sub>A</sub> receptor-mediated and involves activation of mitogen-activated protein (MAP) kinases and perhaps the transactivation of epidermal growth factor receptor (4). There is also direct and indirect evidence suggesting that endothelins stimulate oxidative stress in the vasculature, an effect that some studies have attributed primarily to the ET<sub>A</sub> receptor (21,22). The deleterious vascular effects of endothelins, which have been the subject of much investigation in atherosclerosis, will be described in more detail below.

### **ET<sub>A</sub> and ET<sub>B</sub> receptors in the heart**

Both ET<sub>A</sub> and ET<sub>B</sub> receptors are expressed in a heterogeneous manner throughout the human heart, with ET<sub>A</sub> receptors predominating on myocytes (reviewed by (23)). Endothelins have been reported to exert direct positive inotropic and, less frequently, positive chronotropic effects on the heart under various experimental conditions. The positive inotropic effects are thought to involve activation of protein kinase C and the Na<sup>+</sup>/H<sup>+</sup> exchanger, resulting in

sensitization of the myofilaments to  $\text{Ca}^{2+}$ . The effects of endothelins on normal human cardiac function in vivo are somewhat unclear but may include a tonic  $\text{ET}_A$  receptor-mediated contribution to left ventricular contractility. ET-1 has been shown to increase atrial natriuretic peptide (ANP) mRNA and secretion from isolated atrial myocytes, apparently via  $\text{ET}_A$  receptor activation (24).  $\text{ET}_A$  receptor activation has also been reported to contribute to atrial stretch-induced release of ANP (25). As with vascular smooth muscle cells, endothelins have also been shown to stimulate hypertrophy of cardiac myocytes, an effect which has been shown in neonatal rat cardiac myocytes to be mediated by the  $\text{ET}_A$  receptor and involves the actions of MAP kinases and reactive oxygen species (26).

### **$\text{ET}_A$ and $\text{ET}_B$ Receptors in The Lung**

$\text{ET}_A$  receptors are expressed in the muscular media of human pulmonary arteries with stronger expression in proximal compared to distal arteries (27). The  $\text{ET}_B$  receptor is also found on endothelial cells, the media, and the intima of pulmonary arteries. The expression of the  $\text{ET}_B$  receptor in the intima is higher in proximal than in distal arteries, whereas medial  $\text{ET}_B$  receptor expression is stronger in the distal vessels (27). These data on receptor expression obtained in rats seem to be confirmed by receptor binding studies in human lung tissue (28). Distal arteries possess both more binding sites in the media and a greater proportion of  $\text{ET}_B$  receptors than proximal arteries (28). Furthermore, both  $\text{ET}_A$  and  $\text{ET}_B$  receptors were shown to mediate pulmonary smooth muscle cell proliferation in response to ET-1 (28).

### **$\text{ET}_A$ and $\text{ET}_B$ Receptors in The Kidney**

As reviewed in detail by Kohan (29), most nephron segments can produce and bind endothelins. Autoradiographic studies of human kidneys have shown that binding of endothelin is greatest in the renal medulla, with lower levels of binding observed in the cortex (30,31). The ratios of  $\text{ET}_A$  to  $\text{ET}_B$  receptors are similar in human cortex and medulla, varying from 1:2 to 20:80 depending on the technique used to quantify the receptors (31–33). In general, these receptor distributions are similar among various mammalian species.

The local endothelin system in the kidney plays an important role in the control of blood pressure through their effects on renal  $\text{Na}^+$  and water excretion. In the healthy kidney, endothelins are thought to act as diuretic and natriuretic agents, effects mediated predominantly via  $\text{ET}_B$  receptors. Three main mechanisms are thought to contribute to the natriuretic and diuretic effects of endothelins. Firstly, endothelins inhibit  $\text{Na}^+$  and  $\text{Cl}^-$  transport in several tubular segments, all of which predominantly express  $\text{ET}_B$  receptors (29). Fluid and bicarbonate transport in the proximal tubule is inhibited by endothelins at least partly via suppression of  $\text{Na}^+/\text{K}^+$  ATPase activity (34). Endothelin has been shown to inhibit  $\text{Cl}^-$  reabsorption from cortical and medullary thick ascending limbs, which appears to be mediated, at least in the medulla, by an  $\text{ET}_B$  receptor, NO-mediated mechanism (35,36). There is also evidence that endothelin inhibits  $\text{Na}^+/\text{K}^+$  ATPase activity in the collecting duct, an effect involving cyclooxygenase metabolites (37). Secondly, endothelin has been shown to inhibit vasopressin-induced water reabsorption by the collecting duct via  $\text{ET}_B$  receptor-mediated inhibition of cyclic adenosine monophosphate (cAMP) accumulation (38), although a recent study suggested that  $\text{ET}_A$  receptors located on the collecting duct, albeit present in smaller numbers than  $\text{ET}_B$  receptors, may somehow enhance collecting duct sensitivity to vasopressin (39). Finally,  $\text{ET}_B$  receptor-activation increases renal medullary blood flow via NO and vasodilator cyclooxygenase metabolites (40), a hemodynamic mechanism thought to promote natriuresis. The  $\text{ET}_B$  receptor-dependent enhancement of medullary blood flow may assume even greater importance during high dietary salt intake as rats maintained on a high salt diet display an enhanced  $\text{ET}_B$  receptor-dependent increase of medullary blood flow in response to big ET-1 (41). A functionally intact endothelin system appears essential in order to increase sodium excretion appropriately and maintain a normal blood pressure during increases in

dietary salt intake (17,42,43). This will be discussed further below, as dysfunction of this system is one possible cause of salt-sensitive hypertension.

### ET<sub>B</sub> as a “Clearance” Receptor

Another important function of the ET<sub>B</sub> receptor is its action as a “clearance” receptor for endothelins. Following intravenous injection, ET-1 is rapidly removed from circulation, being retained in tissues (primarily the lungs, kidney and liver), and this effect is inhibited by ET<sub>B</sub> but not ET<sub>A</sub> receptor blockade (44). In further support of ET<sub>B</sub> receptor-mediated clearance of endothelin, plasma ET-1 concentrations are increased during ET<sub>B</sub> receptor blockade (45) and in rats genetically deficient in ET<sub>B</sub> receptors (42). The ability of the ET<sub>B</sub> receptor, but not the ET<sub>A</sub> receptor, to “clear” endothelins is not fully understood because studies have shown that ET<sub>A</sub> receptor selective antagonism can also elevate plasma ET-1 levels (46) and ET<sub>A</sub> binding kinetics are not too dissimilar to those of ET<sub>B</sub> receptors. The endothelin receptors bind ET-1 with extremely high affinity, with the half-life of dissociation from various tissues being > 30 hours in vitro (47). Although some studies have suggested that ET-1-ET<sub>B</sub> receptor complexes may be more stable than ET-1-ET<sub>A</sub> receptor complexes (48), the ET<sub>A</sub> receptor still binds endothelins extremely tight, with a half-time for dissociation of approximately 2 hours at 4°C even at acidic pH (49). Interestingly, this allows intact ET-1 to remain bound to the ET<sub>A</sub> receptor for as long as 2 hours even after the ligand-receptor complex has undergone endocytosis (49). An additional property which may underlie the apparently specific “clearance” function is that once ET<sub>B</sub> receptors bind endothelin, the receptors are internalized and targeted to the late endosomes/lysosomes for degradation, unlike ET<sub>A</sub> receptors which undergo recycling to the plasma membrane (50,51). Finally, another possibility is that ET<sub>B</sub> receptors may simply be more readily accessible to circulating endothelins than are ET<sub>A</sub> receptors, perhaps because ET<sub>B</sub> receptors outnumber ET<sub>A</sub> receptors, or due to the close proximity of the pool of ET<sub>B</sub> receptors located on endothelial cells, resulting in more apparent changes in circulating ET-1 levels when the ET<sub>B</sub> receptor is blocked compared to the ET<sub>A</sub> receptor. Regardless of the mechanism involved, the role of the ET<sub>B</sub> receptor as a clearance receptor for endothelins should obviously be taken into consideration in the context of the treatment of cardiovascular diseases with endothelin receptor antagonists.

### Dimerization of ET<sub>A</sub> and ET<sub>B</sub> receptors

Several unexpected interactions have been reported between ET<sub>A</sub> and ET<sub>B</sub> receptors in cells expressing both subtypes. For example, ET<sub>B</sub> receptors in the anterior pituitary gland appeared to only bind ET-1 during blockade of ET<sub>A</sub> receptors (52). Clearance of ET-1 by astrocytes and production of superoxide by rat aorta is blocked only by a combination of ET<sub>A</sub> and ET<sub>B</sub> receptor antagonists while being unaffected by administration of either agent alone (22,53). Further, either ET<sub>A</sub> or ET<sub>B</sub> selective receptor antagonists can completely block the vasoconstrictor actions of ET-1 in renal afferent arterioles (54).

The existence of ET<sub>A</sub>-ET<sub>B</sub> heterodimers has been offered as a possible explanation for the aforementioned findings and these heterodimers may be the unidentified “atypical” endothelin receptor subtype reported in some studies (52,55–57). Recently it was demonstrated that, at least in transfected cells, ET<sub>A</sub> and ET<sub>B</sub> receptors constitutively form heterodimers and homodimers (58,59). Results thus far suggest that ET<sub>B</sub> receptors may internalize more slowly when present as ET<sub>A</sub>-ET<sub>B</sub> heterodimers, and that the heterodimers only dissociate following internalization after prolonged exposure to ET<sub>B</sub> receptor-selective agonists (58) whereas homodimers appear resistant to ligand-induced dissociation (59). Further studies will hopefully illuminate the functional consequences and significance of hetero- and homodimerization of endothelin receptors.

## EVIDENCE FOR THE UTILITY OF ENDOTHELIN RECEPTOR ANTAGONISTS

The considerable attention being paid to the endothelin system as a therapeutic target by many large and small pharmaceutical firms stems from the potential utility of endothelin receptor antagonists in the treatment of a wide range of cardiovascular diseases. There have been a number of very positive results in a variety of diseases, and in fact, the non-selective antagonist, bosentan, is currently on the market and being used clinically for the treatment of pulmonary arterial hypertension. Despite intensive investigation, there remains considerable controversy as to whether ET<sub>A</sub> selective or non-selective, ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists are preferable. In the end, this decision may be based on the particular disease and the specific pathology of the individual.

## PULMONARY ARTERIAL HYPERTENSION

Pulmonary arterial hypertension (PAH) is a debilitating disease characterized by a sustained increase in pulmonary artery pressure due to elevated pulmonary vascular resistance. The prognosis of patients with untreated PAH is very poor and most patients die within 2–3 years after the diagnosis was made (60). The first significant improvements in prognosis were achieved with vasodilator therapy, such as with calcium channel blockers or prostaglandin administration. However, significant side effects and limited efficacy of these treatments highlight the need for new therapeutic options in this condition.

Circulating ET-1 levels were found to be elevated in patients with PAH, correlating with the severity of the condition (61). In addition, local pulmonary production of ET-1 was found to be strongly increased, both in animal models of PAH (62), in explanted lungs of adult patients with PAH (63) and in lung biopsies of pediatric patients with PAH (64). Pulmonary ET<sub>B</sub> receptors mediate clearance of about 50% of circulating ET-1 (65), and it has been suggested that pulmonary ET-1 clearance may be reduced in patients with PAH. However, it was shown recently that pulmonary clearance of ET-1 is intact in the majority of patients with PAH, indicating that elevated ET-1 levels are mainly due to increased production rather than reduced clearance of circulating ET-1, although reduced clearance in other vascular beds cannot be ruled out (66).

In a hypoxia-induced model of PAH (27), ET<sub>A</sub> receptors in the media of distal arterial vessels were upregulated with no change of ET<sub>B</sub> receptor expression, whereas in the intima, ET<sub>B</sub> receptors were found to be upregulated (27). In contrast, expression of smooth muscle ET<sub>B</sub> receptors were shown to increase over time in an over circulation-induced model of PAH, which was associated with increased ET<sub>B</sub> receptor mediated vasoconstriction (67). In humans, binding sites for both receptor subtypes were found to be up-regulated in distal pulmonary arteries from patients with PAH (28).

In addition to these alterations in protein expression, functional changes of the pulmonary endothelin system have also been observed in models of PAH. The data are conflicting, and initial ET<sub>B</sub> receptor-mediated vasodilatory responses were found to be increased in some studies (68), but reduced in others (69). Likewise, the vasoconstrictor response to ET-1 mediated by smooth muscle ET<sub>A</sub> and ET<sub>B</sub> receptors was found to be enhanced in some (70) and reduced in other studies (71).

Pulmonary veins have attracted more attention recently, because it has been recognized that these post-capillary vessels contribute more to total pulmonary vascular resistance than previously assumed. Depending on the species studied, pulmonary veins contribute up to 50% of total pulmonary vascular resistance (72). ET-1 is a powerful vasoconstrictor of isolated human pulmonary veins, which show increased expression of big ET-1, ET<sub>A</sub> and ET<sub>B</sub> receptors in the media after exposure to hypoxia (73). This is in line with data showing increased ET<sub>B</sub>

receptor-mediated vasoconstriction of pulmonary veins, but not arteries after hypoxia (74). Overall, these data indicate a substantial contribution of the local endothelin system in pulmonary veins to the degree of hypoxia-induced pulmonary hypertension.

Interestingly, recent studies have shown that the signaling pathways utilized by ET-1 might change in rats exposed to prolonged hypoxia. Under normoxic conditions, ET-1 mediated vasoconstriction is largely calcium-dependent. However, the inhibitory effect of L-type calcium channel blockade on ET-1 mediated pulmonary vasoconstriction is reduced after prolonged hypoxia (75). Activation of tyrosine kinases and Rho kinase were recently shown to account for the 'calcium sensitization' of pulmonary arteries exposed to chronic hypoxia (76). Of note, Rho kinase inhibition has been demonstrated to effectively lower pulmonary vascular resistance in patients with PAH (77).

Oxidative stress has been shown to mediate some of the detrimental effects of ET-1 in the pulmonary circulation of the over circulation-induced model of PAH. By activating smooth muscle cell ET<sub>A</sub> receptors and perhaps also ET<sub>B</sub> receptors on endothelial cells, ET-1 increases the production of superoxide and hydrogen peroxide (21,22,78). These reactive oxygen species contribute to the proliferative response of pulmonary vascular smooth muscle cells to ET-1 and inhibit transcription of endothelial nitric oxide synthase and soluble guanylate cyclase (78,79), further increasing pulmonary vascular resistance.

### Endothelin Receptor Blockade in PAH

Endothelin receptor blockade has been uniformly shown to be an effective treatment strategy in a variety of animal models of PAH (Table 1). Two of these studies have directly compared the effectiveness of selective ET<sub>A</sub> receptor blockade versus nonselective ET<sub>A</sub> and ET<sub>B</sub> receptor blockade. In the first study, administration of the ET<sub>A</sub> selective antagonist ABT-627 or combined blockade of both receptors with ABT-627 and A-192621 had similar beneficial effects on right ventricular pressure and hypertrophy in monocrotaline-induced PAH (80). In the second study, the nonselective antagonist BSF420627, but not the ET<sub>A</sub> selective antagonist LU135252 reversed right ventricular hypertrophy and significantly increased survival in the same model (81). Differences in study design may explain the discrepancy of these findings. In the first study, treatment was started before induction of PAH, whereas treatment was started 2 weeks after induction of PAH in the second study, more realistically resembling the clinical scenario.

The non-selective ET antagonist, bosentan, was the first, and currently the only, ET antagonist currently on the market for the treatment of PAH. Food and Drug Administration approval was based in large measure by combined data from two extended clinical trials that showed survival rate of patients treated with bosentan of 86% after three years, as compared to a predicted 48% based on historical data from the National Institutes of Health (60). In addition, it was also shown that bosentan at a dose of 125 mg or lower is effective and safe in children (82).

To date, two clinical trials with newer ET<sub>A</sub> receptor-selective antagonists have been completed. Both sitaxsentan and ambrisentan led to improvements in the 6-minute walk test and several secondary endpoints (83,84). The advantages of ambrisentan seems to be a lower, non-dose dependent incidence of liver toxicity, less interaction with other drugs and the option of once-daily dosing due to a long half-life (9 to 15 hours). In the subsequent phase III ARIES II trial, ambrisentan at doses of 2.5 and 5 mg qd improved exercise capacity without clinically significant increases in liver enzymes (<http://www.myogen.com>).

### Selective ET<sub>A</sub> Receptor Blockade or Nonselective ET<sub>A</sub> and ET<sub>B</sub> Receptor Blockade?

Although ET<sub>B</sub> receptor deficiency has been shown to potentiate hypoxia-induced PAH in rats (85), both selective and nonselective endothelin receptor blockade effectively ameliorate the progression of PAH in clinical trials. So far, no clinical study has made a direct comparison of ET<sub>A</sub> selective versus nonselective ET<sub>A</sub>/ET<sub>B</sub> antagonists in patients with PAH. Based on the role of the renal tubular ET<sub>B</sub> receptor in mediating sodium excretion, differences in the use of diuretics and in the occurrence of edema might be anticipated. Rates of edema were not reported in the any of the bosentan trials, but patients treated with the ET<sub>A</sub> selective receptor antagonist sitaxsentan in the STRIDE-1 trial did not suffer a higher rate of peripheral edema than patients in the placebo group (21% versus 17%, n.s.).

## CHRONIC HEART FAILURE

Circulating levels of ET-1 are elevated in patients with chronic heart failure (CHF) caused by a combination of increased production and reduced clearance of ET-1 (86). Furthermore, activation of the endothelin system contributes to peripheral vasoconstriction and impaired endothelial function in patients with CHF (87), and plasma ET-1 levels were shown to predict survival (88). In the majority of animal models, the expression of ET-1 in the left ventricle was found to be upregulated (89). Some studies found upregulation of either ET<sub>A</sub> or ET<sub>B</sub> receptors, and sometimes both receptors (89–92). In humans, left ventricular expression of ET-1 and ET<sub>A</sub> receptors were found to be increased, with no change in ET<sub>B</sub> receptors (93). Despite the upregulation of ET<sub>A</sub> receptors, the inotropic response to ET-1 is reduced in failing hearts, indicating reduced post-receptor signaling efficiency.

### Endothelin Receptor Blockade in CHF

Most studies in animal models of CHF demonstrated beneficial effects of selective ET<sub>A</sub> receptor blockade on cardiac function and overall mortality, and the same is true for most studies with nonselective receptor blockade (Table 2). Only two of these animal studies compared selective versus nonselective blockade directly. Both studies found that the effects on hemodynamics and cardiac function were similar (94,95).

Early studies showed that endothelin blockade has beneficial hemodynamic effects in humans with CHF. Two recent clinical studies compared the hemodynamic effects of selective versus nonselective endothelin receptor blockade directly. In the first study (96), similar reductions of pulmonary and peripheral vascular resistance and increases in cardiac output were produced by the two types of antagonists, whereas in the second study (97), these effects were found to be more pronounced with selective ET<sub>A</sub> receptor blockade. In both studies, circulating ET-1 levels were only raised by nonselective receptor blockade. The clinical significance of a further increase in plasma ET-1 levels with nonselective blockade is unknown, but may be of minor importance when ET<sub>A</sub> receptors are fully blocked.

The ENCOR trial was the first trial to examine the effects of endothelin receptor blockade in patients with CHF (98). Treatment with the nonselective antagonist, enrasentan, unfortunately led to a deterioration in clinical status and a tendency to increase mortality, as compared with the placebo treated group. A more recent study also showed that enrasentan has adverse effects on left ventricular structure in patients with asymptomatic left ventricular systolic dysfunction (99). In the REACH-1 trial, patients with CHF were treated with bosentan, but the study was interrupted early because of a high incidence of elevated liver enzymes (100). In the subsequent ENABLE 1 and 2 trials, a lower dose of 125 mg bid reduced hepatotoxicity did not improve outcome as compared to placebo (101). Especially in the first 2 weeks of treatment, bosentan led to a high incidence of fluid retention and edema.



So far, two trials have examined the long-term effects of selective ET<sub>A</sub> receptor blockade in patients with CHF. In the HEAT trial, the hemodynamic effects of selective ET<sub>A</sub> receptor blockade with darusentan were determined before and after 3 weeks of treatment (102). Although there was a significant increase in cardiac output with active treatment, major safety concerns were raised following 4 deaths in the darusentan treated group. In addition, this study showed an increase in plasma ET-1 levels at the higher doses of darusentan, indicating that blockade of the ET<sub>A</sub> receptor can increase circulating ET-1 levels in CHF. The EARTH trial failed to detect a beneficial effect of 6 months treatment with darusentan on left ventricular remodeling in patients with CHF (103). At this point, it is not clear why, but the beneficial effects in the acute hemodynamic studies of either selective or non-selective ET antagonists do not appear to translate into clinical benefits during long-term treatment.

### Endothelin Antagonists in Acute Heart Failure

Several small studies have shown beneficial effects of the non-selective ET receptor antagonist, tezosentan, on hemodynamic parameters in patients with acute heart failure. The larger VERITAS trial then examined the effect of tezosentan on the mortality of patients hospitalized with acute heart failure (104). Similar to the situation in chronic heart failure, the trial had to be discontinued prematurely because of lack of a beneficial effect on mortality (104).

### Novel Aspects of Endothelin Action in CHF

A recent study suggested that the effects of endothelin receptor blockade, beneficial or harmful, may crucially depend on the stage of CHF when therapy is initiated (105). These investigators also reported that selective ET<sub>A</sub> receptor blockade during an early stage of CHF caused sustained sodium retention by activating the renin-angiotensin system (105). Another recent study describes a new genetic model of CHF in mice produced by cardiac-specific over-expression of ET-1 (106). The mice suffered cardiac hypertrophy, inflammation, dilation and subsequently death. It has to be noted, however, that the levels of ET-1 in the hearts of the transgenic mice were 10 times greater than in the control mice compared to 3-fold increases reported in humans with CHF (106). Although highly problematic given the negative findings in human CHF, this study raises the question of whether there could be a role for endothelin blockade in inflammatory cardiac disease, such as in the early stages of virally induced cardiomyopathies.

## ESSENTIAL HYPERTENSION

Plasma ET-1 concentrations have been reported to be elevated in patients with essential hypertension in some but not all studies (reviewed in (107)). However, since secretion of ET-1 from endothelial cells is polar, with the majority of ET-1 likely to be secreted towards the media rather than lumen of blood vessels (108), plasma ET-1 concentrations may not give a reliable indication of local vascular exposure to ET-1.

Consistent with a role for ET-1 in hypertension, an enhanced contribution of endogenous ET-1 to forearm vascular tone in patients with essential hypertension has been reported (109). In patients with essential hypertension, forearm vasodilation in response to selective ET<sub>A</sub> receptor blockade or nonselective endothelin receptor blockade was found to be enhanced compared to normotensive subjects (109), although other studies did not find such a difference (19,110). One study directly compared the vasodilatory response of ET<sub>A</sub> versus ET<sub>A</sub>/ET<sub>B</sub> blockade in the forearm vasculature of patients with essential hypertension and found that nonselective blockade had a greater vasodilatory effect than selective ET<sub>A</sub> receptor blockade in these patients (109), perhaps indicating a greater contribution of smooth muscle cell ET<sub>B</sub> receptors to vascular tone or impaired endothelial cell ET<sub>B</sub> receptor-mediated vasodilator production in patients with hypertension.

Despite these encouraging data, few studies have examined the long-term blood pressure lowering potential of endothelin receptor antagonists. In patients with essential hypertension, the non-selective antagonist, bosentan, and the ET<sub>A</sub> antagonist, darusentan, reduce blood pressure to a similar extent as enalapril (111,112). However, these drugs are not used clinically to treat patients with essential hypertension, mainly because of the relatively high liver toxicity, greater incidence of other less severe side effects such as headache and peripheral edema as well as greater costs compared to established anti-hypertensive drugs.

Endothelin receptor blockade may have greater benefits in certain subgroups of patients with hypertension, such as diabetics, African-Americans and obese patients. In these subgroups, the contribution of ET-1 to vascular tone has been shown to be even more pronounced (113–115). Endothelin receptor blockade could also be of value in patients with resistant hypertension, where blood pressure remains uncontrolled despite therapy with 3 or more antihypertensive agents. Darusentan (Myogen) and Thelin (Encysive) are currently being evaluated for the treatment of resistant hypertension.

Although great progress has been made in the treatment of hypertension, blood pressure control is still achieved only in about 30% of patients (116). Control of blood pressure is particularly difficult in elderly subjects with isolated systolic hypertension. In contrast to the hypertension found in younger subjects, isolated systolic hypertension in the elderly is pathogenetically different, being strongly associated with increased arterial stiffness. Agents that interfere with the progression of arterial stiffness might be particularly suited to treat isolated systolic hypertension. In animal experiments, ET-1 has recently been shown to directly increase arterial stiffness, and conversely, ET<sub>A</sub> receptor blockade has been shown to directly decrease arterial stiffness (117). Thus, endothelin antagonists might be useful in the treatment of isolated systolic hypertension through direct effects on the vasculature. Due to the action of the ET<sub>B</sub> receptor to “clear” endothelin and to facilitate renal salt and water excretion (see above), it would appear preferable to treat hypertensive patients with ET<sub>A</sub> receptor antagonists rather than nonselective endothelin receptor antagonists.

Interestingly, some studies suggest that reduced renal production of ET-1 might contribute to the development of hypertension. Several lines of evidence demonstrate that urinary excretion of endothelin specifically reflects renal endothelin production. Renal endothelin production is increased in experimental animals given a high salt diet (17) and changes in sodium excretion are tightly correlated to urinary excretion of ET-1 in humans (118). Patients with hypertension, in particular those with salt-sensitive hypertension, were found to excrete less ET-1 in their urine than normotensive subjects, indicating reduced renal ET-1 production in the hypertensive subjects (119). Reduced production of ET-1 in the renal medulla has also been found in some animal models of hypertension (120,121). Underlining the importance of the intrarenal endothelin system in the control of blood pressure and sodium and water homeostasis, mice with collecting duct-specific knockout of the ET-1 gene are hypertensive on a normal salt diet, and this hypertension is exacerbated by exposure to a high salt diet (43). Further, studies in rats have shown that blockade of ET<sub>B</sub> receptors or genetic ET<sub>B</sub> receptor deficiency leads to salt-sensitive hypertension (17,42). These observations support the hypothesis that a deficiency in renal endothelin production or an impairment of its actions reduces the ability of the kidney to excrete excess Na<sup>+</sup>, leading to the development of salt-sensitive hypertension.

## KIDNEY DISEASE

The renal circulation is particularly sensitive to the effects of intravenous infusion of ET-1 that reduces renal blood flow, glomerular filtration rate (GFR) and sodium excretion in humans in the absence of changed in systemic hemodynamics and is mediated mainly by ET<sub>A</sub> receptors (122). After treatment with the ET<sub>A</sub> receptor antagonist, ABT-627, for one week, no change

was observed in renal hemodynamics, indicating a minor role for endogenous endothelin in the regulation of renal vascular tone in healthy subjects (122).

In patients with chronic kidney disease (CKD), selective blockade of ET<sub>A</sub> receptors increased renal blood flow and reduced blood pressure, filtration fraction, and proteinuria to a greater extent compared to healthy controls (123). Thus, patients with CKD appear to have increased ET<sub>A</sub> receptor-mediated renal vascular tone. In both patients with CKD and healthy controls, ET<sub>B</sub> receptor blockade reduced renal blood flow despite an increase in systemic blood pressure (123). Nonselective endothelin receptor blockade did not change renal blood flow in patients with CKD or in healthy controls (123). Taken together, these data indicate that ET<sub>B</sub> receptors have a predominantly vasodilatory action in the human renal circulation, in healthy subjects and in patients with CKD. Based on these results, selective ET<sub>A</sub> receptor blockade may be more beneficial than nonselective endothelin receptor blockade in patients with CKD.

ET-1 has also been implicated directly in the cellular pathology of several forms of renal disease. The renal endothelin system is activated in autosomal-dominant polycystic kidney disease (ADPKD) and is considered to be a disease-modifying factor (124). ET-1 seems to promote cyst-formation, and furthermore, ET<sub>A</sub> receptor blockade has been shown to increase cyst formation in the Han:SPRD rat, an animal model of ADPKD (125). In most, but not all, models of renal disease, however, selective ET<sub>A</sub> receptor blockade as well as non-selective blockade have both been shown to be beneficial (see Table 3).

Podocytes have attracted increasing attention recently in the context of renal glomerular injury. Podocyte dysfunction leads to breakdown of the glomerular filtration barrier, proteinuria and subsequent kidney damage. ET-1 synthesis is increased in dysfunctional podocytes (126), which promotes contraction of podocytes and neighboring mesangial cells and further increases in protein filtration. ET<sub>A</sub> receptor blockade has recently been shown to reverse established glomerulosclerosis and proteinuria in a model of focal-segmental glomerulosclerosis by more than 50% (127). In addition to podocytes, increased ET production by tubular epithelial cells also has been shown to contribute to renal tubular injury (128). In line with these data, urinary ET-1 excretion is increased in patients with proteinuric kidney disease and decreases after treatment of the underlying renal disease, e.g. by immunosuppressive therapy (129).

With the exception of ADPKD and renal artery stenosis, endothelin receptor blockade may have beneficial effects in most forms of kidney disease although targeted clinical studies in patients with CKD are lacking. In a recent phase II trial, avosentan was shown to reduce proteinuria in patients with diabetic nephropathy by about 30%, even though these patients were already being treated with an angiotensin converting enzyme inhibitor or an angiotensin receptor blocker. The evaluation of avosentan has now moved into a large phase III trial (<http://www.clinicaltrials.gov/ct/show/NCT00120328>), where the long-term effects on morbidity and mortality in patients with diabetic nephropathy will be investigated. Interestingly, a recent preliminary study by Sasser and colleagues demonstrated that 8 week treatment with atrasentan in an animal model of type I diabetes demonstrated reduced proteinuria and reduced renal inflammation (unpublished data). These findings are consistent with studies in heart failure mentioned above where ET<sub>A</sub> blockade reduced inflammation in the heart.

In addition to CKD, blocking the endothelin system may also be beneficial in acute renal failure (ARF). ET-1 and both receptors are upregulated after an ischemic insult to the kidney, in particular in areas of tubular damage (130). Whether the changes in the expression of the endothelin system are beneficial or detrimental for the recovery of kidney structure and function remains ill defined. Several experimental studies have attempted to define the role of endothelin in ARF with most studies reporting beneficial effects of ET receptor blockade

(Table 4). However, one large study investigating the effect of nonselective endothelin receptor blockade with SB 290670 in patients with CKD undergoing coronary angiography reported that patients receiving SB 290670 were more likely to develop radiocontrast-induced ARF than those receiving placebo (131). The interest in endothelin antagonists in the prevention and treatment of ARF has considerably declined after these discouraging results although the question of whether ET<sub>A</sub> receptor-selective compounds may provide benefit has not been adequately investigated.

## ATHEROSCLEROSIS

It has been well documented that vascular endothelin production is elevated in atherosclerosis and influences the development of atherosclerotic lesions through a variety of mechanisms. In forearm blood flow studies in patients with atherosclerosis, there is increased ET<sub>B</sub> receptor dependent vasoconstriction compared to controls (132). Non-selective blockade of endothelin receptors produced a greater increase in forearm blood flow than ET<sub>A</sub> receptor-selective blockade in patients with atherosclerosis, suggesting enhanced ET<sub>B</sub> receptor mediated vasoconstriction (133). However, elevated ET-1 levels have been shown to impair endothelial function, and selective blockade of ET<sub>A</sub> receptors improves endothelium-dependent vasodilation in the forearm circulation of patients with atherosclerosis (134). Since the brachial artery rarely develops atherosclerosis, the pathophysiological significance of these findings is not clear. Furthermore, in patients with coronary artery disease, intracoronary infusion of the ET<sub>A</sub> receptor antagonist, BQ-123, led to vasodilation and local improvement of endothelium-dependent vasodilation (135). This indicates that ET-1 contributes to coronary vascular tone and endothelial dysfunction in patients with coronary artery disease through actions via the ET<sub>A</sub> receptor. The role of coronary ET<sub>B</sub> receptors was not examined in this study.

In human atherosclerotic lesions, enhanced expression of ET<sub>B</sub> receptors in the intima and media was found, particularly in areas underlying an atherosclerotic plaque (136). While this increased expression of smooth muscle cell ET<sub>B</sub> receptors could explain the increased vasoconstrictor effects of sarafotoxin S<sub>6c</sub> in the human forearm vasculature (132), it is tempting to speculate that increased ET<sub>B</sub> receptor expression may be a consequence of increased ET-1 production in an attempt to facilitate clearance of the peptide.

Atherosclerosis is an inflammatory disease, and monocyte/macrophage infiltration of the vasculature is a key event in initiation and progression of atherosclerotic lesions. Endothelin stimulates production of inflammatory cytokines and influences several crucial steps in the inflammatory component of atherosclerosis. This includes increasing the release of various cytokines from monocytes (137) and enhancing the uptake of LDL cholesterol by these cells, promoting a phenotypic change into foam cells (138). ET<sub>B</sub> receptors, but not ET<sub>A</sub> receptors, were found on macrophages that infiltrated atherosclerotic vessels (139). Cytokines released from monocytes/macrophages, in turn, stimulate ET-1 production (140), providing positive feedback for further cytokine production.

Plasma C-reactive protein (CRP) concentration has been shown to be an independent predictor of cardiovascular mortality and may also directly affect the progression of atherosclerosis by upregulating vascular expression of adhesion molecules, cytokines and chemokines. Interestingly, these effects seem to be dependent on the endothelial release of ET-1 (141). Bosentan was shown to inhibit CRP-induced upregulation of ICAM-1, VCAM-1 and MCP-1 on endothelial cells. This effect most likely derives from blockade of ET<sub>B</sub> receptors, the subtype of endothelin receptors found on endothelial cells.

These data overall seem to suggest that ET<sub>B</sub> receptors have predominantly pro-atherosclerotic effects. However, several anti-atherosclerotic effects are also clearly mediated by ET<sub>B</sub> receptors because of its ability to release NO (142). Whether ET<sub>B</sub> receptor blockade is

beneficial or harmful in patients with atherosclerosis is therefore difficult to predict. In several animal models, both ET<sub>A</sub> receptor selective and nonselective endothelin receptor blockade has been shown to inhibit the development of atherosclerotic lesions (138,143–147). No studies so far have compared ET<sub>A</sub> receptor selective and nonselective strategies directly.

#### **SIDE BAR**

##### **Endothelin as a modulator of the sympathetic nervous system?**

Evidence to date suggests that both endothelin receptor subtypes, but particularly the ET<sub>B</sub> receptor, may have complex interactions with the sympathetic nervous system at the pre- and post-junctional level. Studies of vessel-nerve preparations found that endothelins can inhibit electrical stimulus-evoked sympathetic neurotransmitter release, but similar or slightly greater concentrations of ET-1 can also facilitate transmitter release and/or potentiate norepinephrine-induced vasoconstriction (148,149). More recent interest has centered on findings that activation of neuronal ET<sub>B</sub> receptors increases O<sub>2</sub><sup>-</sup> production (150,151). Other studies have provided evidence that reactive oxygen species can enhance peripheral and centrally-mediated sympathetic nerve activity. In direct contrast with this yet-to-be confirmed ET<sub>B</sub> and O<sub>2</sub><sup>-</sup>-mediated stimulatory effect, earlier studies reported that ET<sub>B</sub> receptor activation inhibits norepinephrine release from electrically stimulated renal sympathetic nerves *in vivo*, an effect apparently involving NO (152). While these seemingly disparate findings have yet to be reconciled and more investigation is needed, a combination of several key factors, including the source of the endothelin (e.g. endogenous generation versus pharmacological administration), the cell type(s) harboring the stimulated receptors, and the chemical mediators generated, likely determine the final effects of endothelins on sympathetic function.

#### **SUMMARY**

1. In general, the detrimental vascular effects of ET-1 such as growth and smooth muscle proliferation are mediated by the ET<sub>A</sub> receptor while ET<sub>B</sub> receptors have opposing effects to produce endothelial-dependent vasodilation, promote natriuresis by inhibiting renal sodium reabsorption, and clearing ET-1 from the circulation.
2. However, the question remains whether ET<sub>A</sub> selective or non-selective ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists should be used to treat various clinical conditions because ET<sub>B</sub> receptors on vascular smooth muscle contribute to vasoconstriction in some circumstances and/or locations.
3. Although the expression of both ET<sub>A</sub> and ET<sub>B</sub> receptors in the pulmonary vasculature is increased in pulmonary arterial hypertension, it is not clear whether blocking the ET<sub>B</sub> receptor is beneficial or harmful in this setting since both ET<sub>A</sub> selective and non-selective ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists are beneficial.
4. Surprisingly, clinical trials using either selective or non-selective antagonists for the treatment of heart failure actually produced detrimental effects despite the fact that many studies in animal models have been very promising.
5. Both ET<sub>A</sub> selective and non-selective ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists effectively lower blood pressure in patients with essential hypertension and may improve renal function in diabetic nephropathy. However, vigorous pursuit of these indications has been slow to develop due, in large measure, to the existing availability of highly effective and less expensive antihypertensive drugs.

### UNRESOLVED ISSUES AND FUTURE DIRECTIONS

1. Non-selective ET<sub>A</sub>/ET<sub>B</sub> antagonists are currently being used for the treatment of pulmonary hypertension and selective ET<sub>A</sub> antagonists should be approved soon. This will allow resolution of the hotly debated question of whether one type of antagonist has a clinical advantage over the other.
2. The broad use of endothelin receptor antagonists to treat essential hypertension currently appears unlikely since there is little evidence of an advantage over current therapies. However, future studies may help determine whether these drugs should be used clinically to treat so-called resistant hypertension especially as a co-therapy.
3. Blockade of endothelin receptors has proven to be beneficial in a variety of animal models of other cardiovascular diseases such as atherosclerosis and diabetic nephropathy; whether these promising results translate to the clinic remains to be determined.
4. Elucidating the yet unknown functional consequences of endothelin receptor hetero- and homodimerization should help clarify many physiological and pathophysiological issues related to the endothelin story.
5. Current knowledge of endothelin receptor-specific actions within the sympathetic nervous system is in its infancy, but is expected to be extremely important in modulating cardiovascular function in health and disease.

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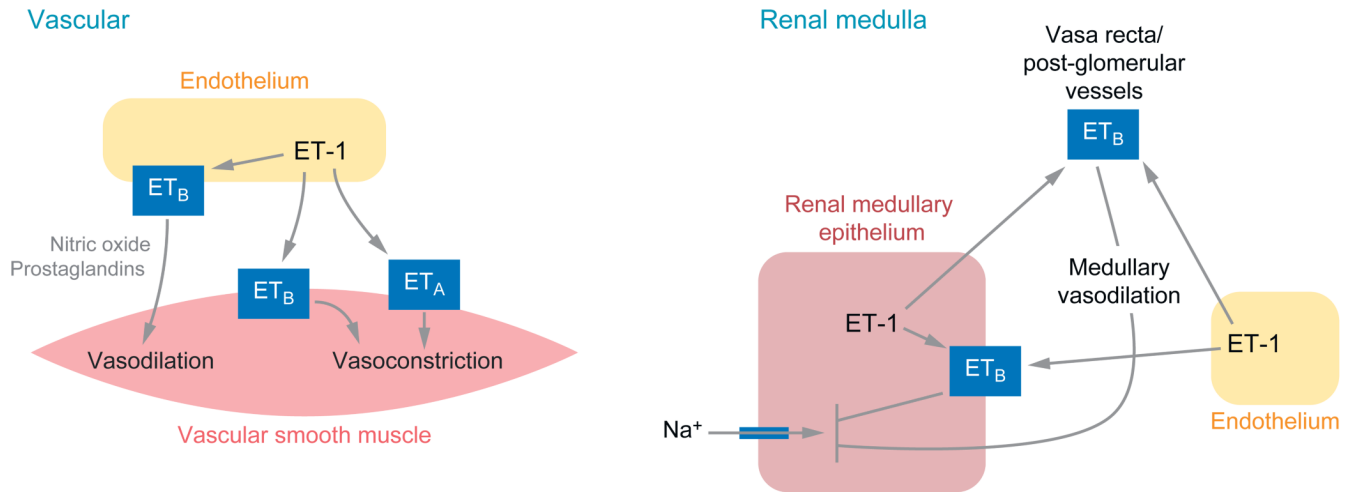
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**Figure 1.**  
Receptor-specific actions of endothelin that influence blood pressure control

Endothelin receptor antagonists used in experimental studies of Pulmonary Arterial Hypertension

**Table 1**

Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (o) no effect (-) harmful	Investigator, Year
RO 47-0203 (Bosentan)	ET <sub>A</sub> & ET <sub>B</sub>	4.7 nM	95 nM	Yes	Overcirculation	+	Rondelet, 2003		
				Yes	Monocrotaline	+	Hill, 1997		
				Yes	Hypoxia	+	Holm, 1996		
				Yes	Hypoxia	+	Chen, 1995		
				Yes	Vascular Study	+	McCulloch, 1998		
SB217242 (Enrasentan)	ET <sub>A</sub>	1.1 nM	1.11 nM	Yes	Hypoxia	+	Underwood, 1998		
				Yes	Hypoxia	+	Underwood, 1997		
				Yes	Hypoxia	+	Underwood, 1999		
				Yes	Vascular Study	+	MacLean, 1998		
SB234551	ET <sub>A</sub>	0.1 nM	500 nM	Yes	Vascular Study	+	MacLean, 1998		
SB209670	ET <sub>A</sub> & ET <sub>B</sub>	0.2 nM	12 nM	No	Vascular Study	+	McCulloch, 1998		
BSF420627	ET <sub>A</sub> & ET <sub>B</sub>	2.2 nM	5.8 nM	Yes	Monocrotaline	+	MacLean, 1998		
BQ123	ET <sub>A</sub>	22 nM	18 μM	No	Overcirculation	+	Jasmin, 2001		
				No	Hypoxia	+	Ivy, 1997		
				No	Hypoxia	+	DiCarlo, 1995		
				No	Hypoxia	+	Oparil, 1995		
				No	Hypoxia	+	Bonvallet, 1994		
				No	Monocrotaline	+	Miyauchi, 1993		
BQ-610	ET <sub>A</sub>	0.7 nM	24 μM	No	Hypoxia	+	Ambalavanan, 2005		
				No	Hypoxia	+	Ambalavanan, 2002		
EMD122946	ET <sub>A</sub>	0.032 nM	160 nM	No	Hypoxia	+	Ambalavanan, 2002		
TBC3711	ET <sub>A</sub>	0.08 nM	26.3 μM	Yes	Hypoxia	+	Perreault, 2001		
TBC11251 (Sitaxsentan)	ET <sub>A</sub>	1.4 nM	9.8 μM	Yes	Monocrotaline	+	Tilton, 2000		
TA-0201	ET <sub>A</sub>	0.015 nM	41 nM	Yes	Monocrotaline	+	Ueno, 2000		
				Yes	Monocrotaline	+	Ueno, 2002		



Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (0) no effect (-) harmful	Investigator, Year
BMS-193884	ET <sub>A</sub>	1.4 nM	18.8 μM	Yes	Monocrotaline	+	Miyuchi, 2000
ZD1611	ET <sub>A</sub>	8.6 nM	5.6 μM	Yes	Hypoxia Hypoxia	+	Bialecki, 1999 Bialecki, 1998
FR 139317	ET <sub>A</sub>	1 nM	7 μM	No	Vascular Study	+	McCulloch, 1998
ABT-627	ET <sub>A</sub>	0.034 nM	63.3 nM	Yes	Monocrotaline	+	Nishida, 2004
<b>(Atrasentan)</b>					Monocrotaline	+	Nishida, 2004
BMS 182874	ET <sub>A</sub>	55 nM	>200 μM	Yes	Vascular Study	+	McCulloch, 1998
YM598	ET <sub>A</sub>	0.697 nM	569 nM	Yes	Hypoxia	+	Yuyama, 2005
					Monocrotaline	+	Yuyama, 2004
					Monocrotaline	+	Fujimori, 2004
LU135252	ET <sub>A</sub>	1.4 nM	140 nM	Yes	Monocrotaline	+	Jasmin, 2003
<b>(Darusentan)</b>					Monocrotaline	+	Jasmin, 2001
					Monocrotaline	+	Prie, 1998
					Monocrotaline	+	Prie, 1997
					Hypoxia	+	Blumberg, 2003
PD-155080	ET <sub>A</sub>	221.4 nM	86.5 μM	Yes	Monocrotaline	+	Stessel, 2004
PD-156707	ET <sub>A</sub>	0.17 nM	133.8 nM	Yes	Overcirculation	+	Fratz, 2004
					Hypoxia	+	Sheedy, 1998
					Hypoxia	+	Haleen, 1998
					Vascular Study	+	Black, 2003

Table 2

Endothelin receptor antagonists used in experimental studies of heart failure

Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (o) no effect (-) harmful	Investigator, Year
RO 47-0203 (Bosentan)	ET <sub>A</sub> & ET <sub>B</sub>	4.7 nM	95 nM	Yes	Coronary ligation	+	Mulder, 1997
RO 61-0612	ET <sub>A</sub> & ET <sub>B</sub>	0.3 nM	10 nM	No	Coronary ligation	+	Oie, 1998
(Tezosentan)					Coronary ligation	+	Fraccarollo, 1997
BQ 123	ET <sub>A</sub>	22 nM	18 μM	No	Coronary ligation	+	Clozel, 2002
SB 209670	ET <sub>A</sub> & ET <sub>B</sub>	0.2 nM	12 nM	No	Coronary ligation	+	Qiu, 2001
YM598	ET <sub>A</sub>	0.697 nM	569 nM	Yes	Coronary ligation	+	Sakai, 1996
ABT-627	ET <sub>A</sub>	0.034 nM	63.3 nM	Yes	Coronary ligation	+	Oie, 2002
(Atrasentan)					Coronary ligation	+	Miyauchi, 2004
LU135252	ET <sub>A</sub>	1.4 nM	140 nM	Yes	J2N-k cardiomyopathy	+	Fujimori, 2004
(Darusentan)					Coronary ligation	+	Nishida, 2005
					ET-1 overexpression	0	Mulder, 2000
					Rapid pacing	-	Yang, 2004
					Coronary ligation	0	Schirger, 2004
					ET-1 overexpression	+	Mulder, 2002
					Rapid pacing	+	Moe, 1998
					Coronary ligation	+	Mulder, 1998
					ET-1 overexpression	0	Fraccarollo, 2002
LU420627	ET <sub>A</sub> & ET <sub>B</sub>	2 nm	6 nm	yes	Coronary ligation	+	Yang, 2004
FR139317	ET <sub>A</sub>	1 nM	7 μM	No	Coronary ligation	-	Nguyen, 2001
TA-0201	ET <sub>A</sub>	0.015 nM	41 nM	Yes	Rapid pacing	+	Chen, 2001
TAK-044	ET <sub>A</sub> & ET <sub>B</sub>	0.08 nM	120 nM	No	Cardiomyopathy	+	Ohnishi, 1998
A-127722	ET <sub>A</sub>	0.082 nM	114 nM	Yes	Rapid pacing	+	Yamauchi-Kohno, 1999
PD 156707	ET <sub>A</sub>	0.17 nM	133.8 nM	Yes	Rapid pacing	+	Ohnishi, 1998
					Rapid pacing	+	Ohnishi, 1998
					Rapid pacing	+	Borgeson, 1998
					Rapid pacing	+	Spinale, 1997

Drug	Selectivity	$E_{T_A}K_i$	$E_{T_P}K_i$	Orally active (yes/no)	Models investigated	Effect (+) beneficial (0) no effect (-) harmful	Investigator, Year
					Rapid pacing	+	McConnell, 2000

**Table 3**  
Endothelin receptor antagonists used in experimental studies of chronic kidney disease

Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (o) no effect (-) harmful	Investigator, Year
RO 47-0203 (Bosentan)	ET <sub>A</sub> & ET <sub>B</sub>	4.7 nM	95 nM	Yes	Ren-2 rat	+	Dvorak, 2004
					Ren-2 rat		Opocensky, 2004
					Ren-2 rat		Vaneckova, 2005
					STZ rat	+	Cosenzi, 2003
					STZ rat	+	Ding, 2003
					Glomerulonephritis 5/6 nephrectomy	+	Gomez-Garre, 1996 Benigni, 1996
RO 48-5695	ET <sub>A</sub> & ET <sub>B</sub>	0.7 nM	5 nM	Yes	5/6 nephrectomy	0	Clozel, 1999
RO 46-2005	ET <sub>A</sub> & ET <sub>B</sub>	220 nM	1 μM	Yes	5/6 nephrectomy	+	Nabokov, 1996
ABT-627 (Atrasentan)	ET <sub>A</sub>	0.034 nM	63.3 nM	Yes	Ren-2 rat	+	Vaneckova, 2005
					Hypokalemia	+	Suga, 2003
LU 224332	ET <sub>A</sub> & ET <sub>B</sub>	3.5 nM	7.2 nM	Yes	Transplant nephropathy	0	Braun, 2000
					Han:SPRD rat	-	Hocher, 2003
					STZ rat	+	Hocher, 2001
LU 302146	ET <sub>A</sub>	0.9 nM	73 nM		Transplant nephropathy	+	Adams, 2002 Knoll, 2003
LU 135252 (Darusentan)	ET <sub>A</sub>	1.4 nM	140 nM	Yes	Transplant nephropathy	+	Braun, 1999 Orth, 1999
					SHR-SP	+	Orth, 1998 Trenkner, 2002
						+	

Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (0) no effect (-) harmful	Investigator, Year
					SHR	+	Brochu, 1999
					5/6 nephrectomy	+	Annann, 2001
					5/6 nephrectomy	-	Hocher, 2003
					Han:SPRD rat	+	Hocher, 2001
					STZ rat	0	Gross, 2003
					STZ rat	+	Dhein, 2000
					Ren-2	0	Rothermund, 2003
					Glomerulosclerosis	+	Ortmann, 2004
					Sabra rat	+	Rothermund, 2003
					SHR/N-cp	+	Gross, 2003
LU 420627	ET <sub>A</sub> & ET <sub>B</sub>	2 nM	6 nM	Yes	Ren-2	0	Rothermund, 2003
BQ-123	ET <sub>A</sub>	22 nM	18 μM	No	2K1C SHR-SP	- +	Hocher, 2000 Okada, 1995
FR 139317	ET <sub>A</sub>	1 nM	7 μM	No	5/6 nephrectomy 5/6 nephrectomy IgA-nephropathy STZ PAN-nephrosis Lupus-nephritis	0 + + + + +	Kohzuki, 1998 Benigni, 1993 Nakamura, 1996 Nakamura, 1995 Ebihara, 1997 Nakamura, 1995
YM 598	ET <sub>A</sub>	0.697 nM	569 nM	Yes	OLETF rats	+	Sugimoto, 2002
PD 142893	ET <sub>A</sub> & ET <sub>B</sub>	31 nM	54 nM	Yes	STZ	+	Benigni, 1998
PD 155080	ET <sub>A</sub>	221.4 nM	86.5 μM	Yes	5/6 nephrectomy	0	Potter, 1997

Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (o) no effect (-) harmful	Investigator, Year
A-127722	ET <sub>A</sub>	0.082 nM	114 nM	Yes	5/6 nephrectomy	0	Pollock, 1997
BMS-182874	ET <sub>A</sub>	55 nM	>200 μM	Yes	5/6 nephrectomy	+	Nabokov, 1996

Table 4

Endothelin receptor antagonists used in experimental studies of acute renal failure

Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (o) no effect (-) harmful	Investigator, Year
RO 47-0203 (Bosentan)	ET <sub>A</sub> & ET <sub>B</sub>	4.7 nM	95 nM	Yes	Post-ischemia Rhabdomyolysis	+ +	Jerkic, 2004 Karam, 1995
RO 61-0612 (Tezosentan)	ET <sub>A</sub> & ET <sub>B</sub>	0.3 nM	10 nM	No	Post-ischemia	+	Wilhelm, 2001
LU 135252 (Darusentan)	ET <sub>A</sub>	1.4 nM	140 nM	Yes	Transplant rejection Post-ischemia Post-ischemia	+ + +	Braun, 2000 Knoll, 2001 Birck, 1998
SB 234551	ET <sub>A</sub>	0.13 nM	500 nM	Yes	Post-ischemia	+	Forbes, 2001
SB 209670	ET <sub>A</sub> & ET <sub>B</sub>	0.2 nM	12 nM	No	Post-ischemia Post-ischemia Post-ischemia Post-ischemia Post-ischemia Post-ischemia Radiocontrast Endotoxin	+ - 0 + + + + 0	Huang, 2002 Forbes, 2001 Forbes, 1999 Brooks, 1994 Gellai, 1995 Ajis, 2003 Brooks, 1996 Wellings, 1995
UK-350926	ET <sub>A</sub>	0.1 nM		No	Post-ischemia Post-ischemia	+ -	Huang, 2002 Ajis, 2003
ABT-627	ET <sub>A</sub>	0.034 nM	63.3 nM	Yes	Post-ischemia	+	Kuro, 2000
PD-156707	ET <sub>A</sub>	0.17 nM	133.8 nM	Yes	Post-ischemia	0	Forbes, 1999

Drug	Selectivity	ET <sub>A</sub> K <sub>i</sub>	ET <sub>B</sub> K <sub>i</sub>	Orally active (yes/no)	Models investigated	Effect (+) beneficial (0) no effect (-) harmful	Investigator, Year
A-127722	ET <sub>A</sub>	0.082 nM	114 nM	Yes	Radiocontrast	+	Pollock, 1997
L-754142	ET <sub>A</sub> & ET <sub>B</sub>	0.062nM	2.25 nM	Yes	Post-ischemia	+	Krause, 1997
BMS-182874	ET <sub>A</sub>	55 nM	>200 μM	Yes	Radiocontrast	+	Bird, 1996
BQ-123	ET <sub>A</sub>	22 nM	18 μM	No	Post-ischemia	0	Brooks, 1994
					Post-ischemia	+	Gellai, 1994
					Post-ischemia	+	Chan, 1994
					Cyclosporine	+	Fogo, 1992
TAK-044	ET <sub>A</sub> & ET <sub>B</sub>	0.08 nM	120 nM	No	Post-ischemia	+	Kusumoto, 1994