Contrasting Actions of Endothelin ET_A and ET_B Receptors in Cardiovascular Disease

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Key Words

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

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_A and ET_B, which usually have opposing actions, mediate the actions of endothelin. ET_A receptors function to promote vasoconstriction, growth, and inflammation, whereas ET_B 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_B 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_A-selective or nonselective ET_A/ET_B receptor antagonists would be useful in a range of clinical settings.

INTRODUCTION

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. 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 -nonspecific 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, this review focuses 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 proinflammatory 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). Preproendothelins are converted within the cell first to the inactive proendothelin peptide after removal of the signal peptide and then into 38 to 41-amino-acid-long "big endothelins," a step catalyzed by furin in the case of ET-1. The big endothelins then

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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 subisoforms of both ECE-1 and ECE-2, with these subisoforms differing in subcellular 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 in 5, 6).

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, ^{125}I-labeled ET-1 was displaced from one receptor by all three peptides, with ET-1 displaying the highest potency (7), whereas all three isopeptides displayed similar potencies in displacing ^{125}I-labeled ET-1 from the other receptor (8). These two receptors are what are now known as the ET_A and ET_B receptors, respectively, and are classified on the basis of their rank order of potencies for the endothelins, being ET-1 = ET-2 $\gg$ ET-3 for the ET_A receptor and ET-1 = ET-2 = ET-3 for the ET_B 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) (**Figure 1**).

The amino acid sequences deduced from cloned ET_A and ET_B receptors predict that these receptors are heptahelical G protein–coupled receptors. In humans, the

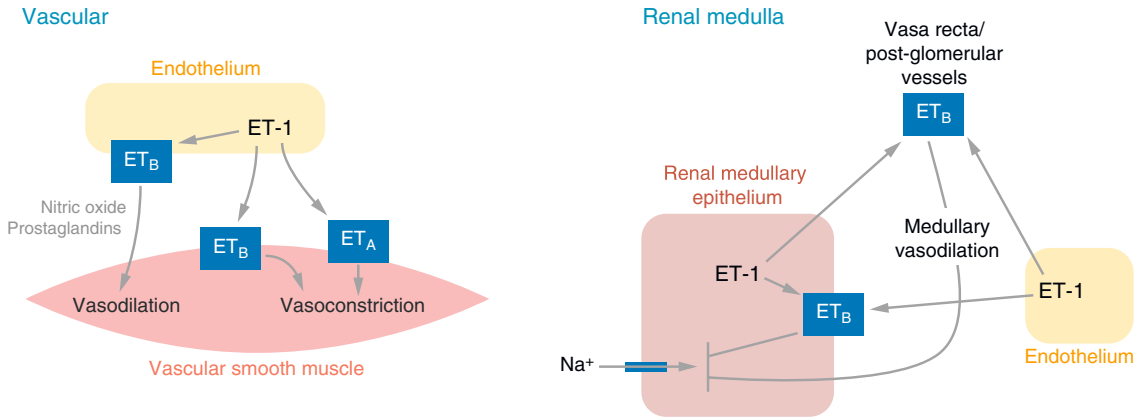

Figure 1

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

ET_A receptor is predicted to be 427 amino acids long, and shows approximately 64% sequence similarity to the predicted 442-amino-acid-long human ET_B receptor (12). Endothelin receptors from several mammalian species display fairly high degrees of homology with the human ET_A receptor (>90%) and ET_B 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 & Sokolovsky (14) demonstrated, using fibroblasts overexpressing either one or the other of the two receptor subtypes, that ET_A and ET_B receptors couple to multiple classes of G-proteins, and that this coupling varies depending on 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_A and ET_B Receptors in the Vasculature

Both ET_A and ET_B receptors located on vascular smooth muscle mediate the potent vasoconstrictor effects that are characteristic of the endothelins (3) (**Figure 1**). 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 (Ca^{2+}) mobilization from intra- and extracellular sources are involved. The ET_B receptor exerts a dual role on vascular tone, as activation of ET_B 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) (**Figure 1**). The predominant influence of endogenous endothelins on vascular tone and basal blood pressure is somewhat contentious. Acute ET_A receptor blockade produces either a small decrease or no change in mean arterial pressure (15), perhaps suggesting that ET_A receptors play relatively little role in determining basal vascular tone in healthy individuals. In contrast, acute and chronic ET_B receptor blockade has consistently been shown to increase mean arterial pressure (16, 17), an effect that appears to involve enhanced ET_A receptor activation but is also compounded by the loss of ET_B receptor-mediated NO production. Thus it could be argued that the ET_B receptor plays a more important role in the control of basal blood pressure and vascular tone than the ET_A 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. The demonstration of increases in forearm blood flow in response to local infusion of the ECE inhibitor phosphoramidon (18) or nonselective endothelin receptor blockade (19) supports this contention. Further, systemic administration of combined ET_A and ET_B antagonists has been shown to produce mild decreases in total peripheral resistance and mean arterial pressure in healthy humans (20).

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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_A 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_A receptor (21, 22). The deleterious vascular effects of endothelins, which have been the subject of much investigation in atherosclerosis, are described in more detail below.

ET_A and ET_B Receptors in the Heart

Both ET_A and ET_B receptors are expressed in a heterogeneous manner throughout the human heart, with ET_A receptors predominating on myocytes (reviewed in 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^+/H^+ exchanger, resulting in sensitization of the myofilaments to Ca^{2+}. The effects of endothelins on normal human cardiac function in vivo are somewhat unclear but may include a tonic 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 ET_A receptor activation (24). 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 that has been shown in neonatal rat cardiac myocytes to be mediated by the ET_A receptor and involves the actions of MAP kinases and reactive oxygen species (26).

ET_A and ET_B Receptors in the Lung

ET_A receptors are expressed in the muscular media of human pulmonary arteries with stronger expression in proximal compared to distal arteries (27). The ET_B receptor is also found on endothelial cells, the media, and the intima of pulmonary arteries. The expression of the ET_B receptor in the intima is higher in proximal than in distal arteries, whereas medial 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 ET_B receptors than proximal arteries (28). Furthermore, both ET_A and ET_B receptors were shown to mediate pulmonary smooth muscle cell proliferation in response to ET-1 (28).

ET_A and 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

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of endothelin is greatest in the renal medulla, with lower levels of binding observed in the cortex (30, 31). The ratios of ET_A to 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 its effects on renal sodium (Na^+) and water excretion. In the healthy kidney, endothelins are thought to act as diuretic and natriuretic agents, effects mediated predominantly via ET_B receptors. Three main mechanisms are thought to contribute to the natriuretic and diuretic effects of endothelins (**Figure 1**). First, endothelins inhibit Na^+ and Cl^- transport in several tubular segments, all of which predominantly express ET_B receptors (29). Fluid and bicarbonate transport in the proximal tubule is inhibited by endothelins at least partly via suppression of Na^+/K^+ ATPase activity (34). Endothelin has been shown to inhibit Cl^- reabsorption from cortical and medullary thick ascending limbs, which appears to be mediated, at least in the medulla, by an ET_B receptor, nitric oxide (NO)-mediated mechanism (35, 36). There is also evidence that endothelin inhibits Na^+/K^+ ATPase activity in the collecting duct, an effect involving cyclooxygenase metabolites (37). Second, endothelin has been shown to inhibit vasopressin-induced water reabsorption by the collecting duct via ET_B receptor-mediated inhibition of cyclic adenosine monophosphate (cAMP) accumulation (38), although a recent study suggested that ET_A receptors located on the collecting duct, albeit present in smaller numbers than ET_B receptors, may somehow enhance collecting duct sensitivity to vasopressin (39). Finally, ET_B receptor activation increases renal medullary blood flow via NO and vasodilator cyclooxygenase metabolites (40), a hemodynamic mechanism thought to promote natriuresis. The 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 ET_B receptor-dependent increase of medullary blood flow in response to big ET-1 (41). A functionally intact endothelin system appears essential to increase Na^+ excretion appropriately and maintain a normal blood pressure during increases in dietary salt intake (17, 42, 43). This is discussed below, as dysfunction of this system is one possible cause of salt-sensitive hypertension.

ET_B as a "Clearance" Receptor

Another important function of the ET_B receptor is its action as a "clearance" receptor for endothelins. Following intravenous injection, ET-1 is rapidly removed from circulation and retained in tissues (primarily the lungs, kidney, and liver), and this effect is inhibited by ET_B but not ET_A receptor blockade (44). In further support of ET_B receptor-mediated clearance of endothelin, plasma ET-1 concentrations are increased during ET_B receptor blockade (45) and in rats genetically deficient in ET_B receptors (42). The ability of the ET_B receptor, but not the ET_A receptor, to "clear" endothelins is not fully understood because studies have shown that ET_A receptor

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selective antagonism can also elevate plasma ET-1 levels (46) and ET_A binding kinetics are not too dissimilar to those of ET_B receptors. The endothelin receptors bind ET-1 with extremely high affinity, with the half-life of dissociation from various tissues being >30 h in vitro (47). Although some studies have suggested that ET-1-ET_B receptor complexes may be more stable than ET-1-ET_A receptor complexes (48), the ET_A receptor still binds endothelins extremely tightly, with a half-time for dissociation of approximately 2 h at 4°C even at acidic pH (49). Interestingly, this allows intact ET-1 to remain bound to the ET_A receptor for as long as 2 h even after the ligand-receptor complex has undergone endocytosis (49). An additional property that may underlie the apparently specific "clearance" function is that once ET_B receptors bind endothelin, the receptors are internalized and targeted to the late endosomes/lysosomes for degradation, unlike ET_A receptors that undergo recycling to the plasma membrane (50, 51). Finally, another possibility is that ET_B receptors may simply be more readily accessible to circulating endothelins than are ET_A receptors, perhaps because ET_B receptors outnumber ET_A receptors or owing to the close proximity of the pool of ET_B receptors located on endothelial cells, resulting in more apparent changes in circulating ET-1 levels when the ET_B receptor is blocked compared to the ET_A receptor. Regardless of the mechanism involved, the role of the ET_B 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.

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Dimerization of ET_A and ET_B Receptors

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

The existence of ET_A-ET_B 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_A and ET_B receptors constitutively form heterodimers and homodimers (58, 59). Results thus far suggest that ET_B receptors may internalize more slowly when present as ET_A-ET_B heterodimers and that the heterodimers only dissociate following internalization after prolonged exposure to ET_B 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.

ENDOTHELIN AS A MODULATOR OF THE SYMPATHETIC NERVOUS SYSTEM?

Evidence to date suggests that both endothelin receptor subtypes, but particularly the ET_B receptor, may have complex interactions with the sympathetic nervous system at the pre- and postjunctional 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_B receptors increases O_2^- 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_B and O_2^--mediated stimulatory effect, earlier studies reported that ET_B receptor activation inhibits norepinephrine release from electrically stimulated renal sympathetic nerves in vivo, an effect apparently involving NO (152). Although 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 determines the final effects of endothelins on sympathetic function.

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 nonselective antagonist bosentan is currently on the market and being used clinically for the treatment of pulmonary arterial hypertension (PAH). Despite intensive investigation, there remains considerable controversy as to whether ET_A-selective or nonselective, ET_A/ET_B 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

PAH is a debilitating disease characterized by a sustained increase in pulmonary artery pressure owing to elevated pulmonary vascular resistance. The prognosis of patients with untreated PAH is very poor and most patients die within 2 to 3 years after

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diagnosis (60). The first significant improvements in prognosis were achieved with vasodilator therapy, such as with Ca^{2+} 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 are 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 in animal models of PAH (62), explanted lungs of adult patients with PAH (63), and lung biopsies of pediatric patients with PAH (64). Pulmonary ET_B receptors mediate clearance of approximately 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_A receptors in the media of distal arterial vessels were upregulated with no change of ET_B receptor expression, whereas in the intima, ET_B receptors were found to be upregulated (27). In contrast, expression of smooth muscle ET_B receptors was shown to increase over time in an overcirculation-induced model of PAH, which was associated with increased ET_B receptor-mediated vasoconstriction (67). In humans, binding sites for both receptor subtypes were found to be upregulated 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 endothelial ET_B 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_A and ET_B receptors was found to be enhanced in some studies (70) and reduced in others (71).

Pulmonary veins have attracted more attention recently because it has been recognized that these postcapillary 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_A, and ET_B receptors in the media after exposure to hypoxia (73). This is consistent with data showing increased ET_B 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 Ca^{2+}-dependent. However, the inhibitory effect of L-type Ca^{2+}-channel blockade on ET-1-mediated pulmonary vasoconstriction is reduced after prolonged hypoxia (75). Activation of tyrosine

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kinases and Rho kinase was recently shown to account for the Ca^{2+} 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 overcirculation-induced model of PAH. By activating smooth muscle cell ET_A receptors and perhaps also ET_B 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 NO 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 [see **Supplemental Table 1** (follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**)]. Two of these studies have directly compared the effectiveness of selective ET_A receptor blockade with nonselective ET_A/ET_B receptor blockade. In the first study, administration of the ET_A-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_A-selective antagonist LU135252, reversed right ventricular hypertrophy and significantly increased survival in the same model (81). Differences in study design may explain the discrepancy between 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 nonselective ET_A/ET_B antagonist bosentan was the first, and is currently the only, endothelin antagonist on the market for the treatment of PAH. Food and Drug Administration approval was based in large measure on combined data from two extended clinical trials that showed a 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_A receptor-selective antagonists have been completed. Both sitaxsentan and ambrisentan led to improvements in the 6-min walk test and several secondary endpoints (83, 84). The advantages of ambrisentan seem to be a lower, nondose-dependent incidence of liver toxicity, less interaction with other drugs, and the option of once-daily dosing owing to a long half-live (9 to 15 h). In the subsequent Phase III ARIES II trial, ambrisentan at doses of 2.5 and 5 mg per day improved exercise capacity without clinically significant increases in liver enzymes (**http://www.myogen.com**).

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Selective ET_A Receptor Blockade or Nonselective ET_A/ET_B Receptor Blockade?

Although ET_B 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_A-selective versus nonselective ET_A/ET_B antagonists in patients with PAH. Based on the role of the renal tubular ET_B receptor in mediating Na^+ 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_A-selective receptor antagonist sitaxsentan in the STRIDE-1 trial did not experience 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_A or ET_B receptors, and sometimes both receptors (89–92). In humans, left ventricular expression of ET-1 and ET_A receptors was found to be increased, with no change in ET_B receptors (93). Despite the upregulation of ET_A receptors, the inotropic response to ET-1 is reduced in failing hearts, indicating reduced postreceptor signaling efficiency.

Endothelin Receptor Blockade in CHF

Most studies in animal models of CHF demonstrated beneficial effects of selective ET_A receptor blockade on cardiac function and overall mortality, and the same is true for most studies with nonselective receptor blockade (**Supplemental 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_A 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_A receptors are fully blocked.

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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 placebo. 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 twice daily reduced hepatotoxicity, but did not improve the outcome as compared with 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_A receptor blockade in patients with CHF. In the HEAT trial, the hemodynamic effects of selective ET_A 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 four 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_A receptor can increase circulating ET-1 levels in CHF. The EARTH trial failed to detect a beneficial effect of 6 months of 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 nonselective ET_A/ET_B 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 nonselective ET_A/ET_B 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). This study also reported that selective ET_A receptor blockade during an early stage of CHF caused sustained Na^+ retention by activating the renin-angiotensin system (105). Another recent study describes a new genetic model of CHF in mice produced by cardiac-specific overexpression of ET-1 (106). The mice suffered cardiac hypertrophy, inflammation, dilation, and subsequently death. It must 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 threefold increases reported in humans

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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, because secretion of ET-1 from endothelial cells is polar, with the majority of ET-1 likely to be secreted toward 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_A 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_A versus ET_A/ET_B blockade in the forearm vasculature of patients with essential hypertension and found that nonselective blockade had a greater vasodilatory effect than selective ET_A receptor blockade (109), perhaps indicating a greater contribution of smooth muscle cell ET_B receptors to vascular tone or impaired endothelial cell ET_B 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 nonselective antagonist bosentan and the ET_A 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 antihypertensive 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 three 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 approximately 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

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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_A 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_B receptor to "clear" endothelin and to facilitate renal salt and water excretion (see above), it would appear preferable to treat hypertensive patients with ET_A receptor antagonists rather than nonselective ET_A/ET_B 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 Na^+ 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 Na^+ 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_B receptors or genetic ET_B 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^+, 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, which reduces renal blood flow, glomerular filtration rate (GFR), and Na^+ excretion in humans in the absence of changes in systemic hemodynamics and is mediated mainly by ET_A receptors (122). After treatment of healthy humans with the ET_A 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_A 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_A receptor-mediated renal vascular tone. In patients with CKD and healthy controls, ET_B receptor blockade reduced renal blood flow despite an increase in systemic blood pressure (123). Nonselective ET_A/ET_B receptor blockade did not change renal blood flow in patients with CKD or in healthy controls (123). Taken together, these data indicate that ET_B receptors have a

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predominantly vasodilatory action in the human renal circulation in healthy subjects and patients with CKD. Based on these results, selective ET_A 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 a disease-modifying factor (124). ET-1 seems to promote cyst formation, and furthermore, ET_A receptor blockade has been shown to increase cyst formation in the Han:SPRD rat, an animal model of ADPKD, perhaps indicating that cyst formation is mediated by the ET_B receptor (125). In most, but not all, models of renal disease, however, selective ET_A receptor blockade as well as nonselective ET_A/ET_B blockade have both been shown to be beneficial (see **Supplemental 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, leading to further increases in protein filtration. ET_A 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-1 production by tubular epithelial cells also has been shown to contribute to renal tubular injury (128). Consistent 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 approximately 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**), in which 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 reduced proteinuria and reduced renal inflammation (J.M. Sasser, unpublished data). These findings are consistent with the studies of heart failure mentioned above, where ET_A 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

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beneficial effects of endothelin receptor blockade (**Supplemental Table 4**). However, one large study investigating the effect of nonselective endothelin receptor blockade with SB 29,0670 in patients with CKD undergoing coronary angiography reported that patients receiving SB 29,0670 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_A 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_B receptor–dependent vasoconstriction compared to controls (132). Nonselective blockade of endothelin receptors produced a greater increase in forearm blood flow than ET_A receptor–selective blockade in patients with atherosclerosis, suggesting enhanced ET_B receptor–mediated vasoconstriction (133). However, elevated ET-1 levels have been shown to impair endothelial function, and selective blockade of ET_A receptors improves endothelium-dependent vasodilation in the forearm circulation of patients with atherosclerosis (134). Because 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_A 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_A receptor. The role of coronary ET_B receptors was not examined in this study.

In human atherosclerotic lesions, enhanced expression of ET_B receptors in the intima and media was found, particularly in areas underlying an atherosclerotic plaque (136). Although this increased expression of smooth muscle cell ET_B receptors could explain the increased vasoconstrictor effects of sarafotoxin S6c in the human forearm vasculature (132), it is tempting to speculate that increased ET_B 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_B receptors, but not ET_A 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.

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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_B receptors, the subtype of endothelin receptors present on endothelial cells.

These data overall seem to suggest that ET_B receptors have predominantly proatherosclerotic effects. However, several antiatherosclerotic effects are also clearly mediated by the ET_B receptor because of its ability to stimulate NO production (142). Whether ET_B receptor blockade is beneficial or harmful in patients with atherosclerosis is therefore difficult to predict. In several animal models, both ET_A receptor–selective and nonselective ET_A/ET_B receptor blockade have been shown to inhibit the development of atherosclerotic lesions (138, 143–147). So far, no studies have compared ET_A receptor-selective and nonselective strategies directly.

SUMMARY POINTS

1. In general, the detrimental vascular effects of ET-1, such as smooth muscle growth and proliferation, are mediated by the ET_A receptor, whereas ET_B receptors have opposing effects to produce endothelium-dependent vasodilation, promote natriuresis by inhibiting renal Na^+ reabsorption, and clearing ET-1 from the circulation.
2. However, the question remains whether ET_A-selective or nonselective ET_A/ET_B receptor antagonists should be used to treat various clinical conditions because ET_B receptors on vascular smooth muscle contribute to vasoconstriction in some circumstances and/or locations.
3. Although the expression of both ET_A and ET_B receptors in the pulmonary vasculature is increased in pulmonary arterial hypertension, it is not clear whether blocking the ET_B receptor is beneficial or harmful in this setting because both ET_A-selective and nonselective ET_A/ET_B receptor antagonists are beneficial.
4. Surprisingly, clinical trials using either selective or nonselective 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_A-selective and nonselective ET_A/ET_B 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.

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UNRESOLVED ISSUES AND FUTURE DIRECTIONS

1. Nonselective ET_A/ET_B antagonists are currently being used for the treatment of pulmonary hypertension, and selective ET_A 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 because 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 resistant hypertension, especially in combination with other antihypertensive agents.
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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