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Endothelin

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Abstract

Endothelin-1 is the most potent vasoconstrictor agent currently identified, and it was originally isolated and characterized from the culture media of aortic endothelial cells. Two other isoforms, termed endothelin-2 and endothelin-3, were subsequently identified, along with structural homologues isolated from the venom of *Actractapis eng-addensis* known as the sarafotoxins. In this review, we will discuss the basic science of endothelins, endothelin-converting enzymes, and endothelin receptors. Only concise background information pertinent to clinical physician is provided. Next we will describe the pathophysiological roles of endothelin-1 in pulmonary arterial hypertension, heart failure, systemic hypertension, and female malignancies, with emphasis on ovarian cancer. The potential intervention with pharmacological therapeutics will be succinctly summarized to highlight the exciting pre-clinical and clinical studies within the endothelin field. Of note is the rapid development of selective endothelin receptor antagonists, which has led to an explosion of research in the field.

Keywords

Endothelin; Endothelin-converting enzymes; Endothelin receptor; Endothelin signal; Pathophysiology

Introduction

The main purpose of this review is to provide a broad overview on the basic science of the endothelin system and its clinical relevance. In the basic science portions of the review, we will begin our discussion on the synthesis of endothelins, endothelin-converting enzymes, and endothelin receptors. For our clinical discussion, we will describe the pathophysiological intervention of pulmonary arterial hypertension with regard to the endothelin system. We will also visit much-discussed topics of endothelin in heart failure, systemic hypertension, and ovarian cancer. Other clinical interventions and diseases within the context of endothelin have also been suggested, and we will conclude our discussion with future possibilities for endothelin antagonist therapy.

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Endothelins

The human genes of endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3) are located on chromosomes 6, 1, and 20, respectively. Endothelin-1 expression is determined primarily at the level of gene transcription regulated by a promoter region located upstream (5′) of the preproendothelin-1 gene. A binding site of GATA mediates basal levels of gene transcription of preproendothelin-1 gene. Ap-1 nuclear factor and a hexonucleotide sequence that control gene transcription are thought to be regulated by angiotensin II, transforming growth factor beta, and/or acute phase reactants. Further post-transcriptional modulation occurs via selective destabilization of preproendothelin-1 mRNA via 'suicide motifs' present in the non-translated 3′ region. This may account for a short, 15-min half-life of preproendothelin-1 mRNA and thereby prevent excessive endothelin-1 production. Factors known to promote endothelin-1 production include thrombin, insulin, cyclosporine, epinephrine, angiotensin II, cortisol, inflammatory mediators, hypoxia, and vascular shear stress. Endothelin production is inhibited by nitric oxide, nitric oxide donor drugs, and dilator prostanoids via an increase in cellular cGMP, and natriuretic peptides via an increase in cAMP levels [1]. The mature endothelin-1 peptide is generated by enzymatic cleavage of the initial preproendothelin-1 gene product (Fig. 1). A short hydrophobic secretory sequence is first removed to produce proendothelin-1, which is further cleaved at dibasic amino acid pairs by the endopeptidase furin generating the 39-amino acid peptide big endothelin-1 [2]. Subsequent production of mature endothelin-1 by a proteolytic cleavage between Trp^{21} and Val^{22} is catalyzed by the membrane bound metalloprotease endothelin-converting enzyme-1 (ECE-1) [3]. Although additional ECE isoforms have been identified in animals, a human ECE-2 and ECE-3 have yet to be identified [4]. ECE gene knockout studies suggest that ECE-1 is the major functional ECE for all three endothelin isoforms in vivo [5]. Endothelin-1 was initially considered to be produced de novo in response to the factors described earlier. However, secretory vesicles containing both mature endothelin-1 and ECE have been identified in endothelial cells [6]. Recently, a new endothelin peptide with 31 amino acids has been identified in humans. This endothelin is formed through the cleavage of the big endothelin-1 between the Tyr^{31} and Gly^{32} amino acids by a human chymase enzyme expressed in mast cells. This product has been termed endothelin-$1_{1–31}$ [7]. Endothelin-$1_{1–31}$ triggered pressor responses that were reduced by endothelin receptor antagonists. These pressor responses to endothelin-$1_{1–31}$ were abolished by the neutral endopeptidase inhibitor thiorphan, but were unaffected by the endothelin-converting enzyme inhibitor CGS35066 [8]. Each of the three endothelin peptides is expressed in various tissues and cells. ET-1 is produced by vascular endothelial and smooth muscle cells, airway epithelial cells, macrophages, fibroblasts, cardiac myocytes, brain neurons, and pancreatic islets [3, 9]. ET-2 is expressed in the ovary and intestinal epithelial cells [3]. ET-3 is found in endothelial cells and intestinal epithelial cells. ET-3 mediates release of vasodilators, including NO and prostacyclin [3].

Endothelin-converting enzyme

ECE-1 was first isolated and purified from aortic endothelial cells [10]. It is inhibited by the combined ECE and neutral endopeptidase (NEP) inhibitor phosphoramidon or selective ECE inhibitor CGS35066, but not by selective NEP inhibitors such as thiorphan or kelatorphan [11]. Structurally, ECE-1 exists as a transmembrane 758-amino acid dimer, linked by a single disulphide bridge. A short (1–56) N-terminal intracellular region is connected by a 21-amino acid transmembrane portion. ECE-1 belongs to a family of neutral metalloprotease enzymes, which includes NEP and the human Kell blood group proteins [12]. However, ECE is unique amongst this group in that it recognizes a relatively long C-terminal portion of big endothelin-1 (residues His27 to Gly34) in addition to the cleavage site between residues 21 and 22 [13]. The ECE-1 gene is located on chromosome 1 at the

p36 band [14]. cDNA cloning studies have demonstrated that differential gene splicing leads to the production of four isoforms of ECE-1, termed ECE-1a, ECE-1b, ECE-1c, and ECE-1d, which differ in structure only at the N-terminus. ECE-1a is responsible for generating the majority of functional endothelin-1 from big endothelin-1. ECE-1a is expressed by endothelial cells and is located intracellularly. The enzymatically active C-terminal segment faces the intra-luminal region of the Golgi apparatus. A generator role for ECE-1a is further suggested by the presence of characteristic promoter regions for this gene, indicating that it is a constitutively expressed 'housekeeping' gene. In contrast, ECE-1b spans the plasma membrane of effector cells, such as vascular smooth muscle cells, converting extracellular big endothelin-1 to endothelin-1. A 'responder/regulator' role for ECE-1b to extracellular big endothelin-1 is suggested from its promoter region containing potential receptor sites for transcription factors, allowing modulation activity. ECE-1c contributes to the elevation of ET-1 peptide levels in diabetes. In particular, expression of ECE-1c seems to respond to high glucose levels in endothelial cells [15]. It is also speculated that different ECE-1 isoforms may be responsible for different cellular functions in cancers. For example, transient ECE-1c overexpression increased cancer invasiveness through Matrigel™, whereas transient ECE-1a expression suppressed invasion. In addition, transient ECE-1a expression in stromal cells strongly counteracts the effect of transient ECE-1c expression in cancer cells. Thus, it is concluded that ECE-1a and ECE-1c are significant, but with reciprocal effects on cell invasion [16].

Transfection of preproendothelin-1 and ECE-1b genes into cultured cells demonstrates that ECE-1b expressed at the cell surface is relatively inefficient at proteolysis of exogenous big endothelin-1, with only around 10% converted to endothelin-1. On the other hand, between 50 and 90% of the endothelin peptides secreted are in the mature endothelin-1 form [12], which suggests that endogenously generated endothelin-1 secreted luminally is the most functionally important source and confirms a predominantly autocrine/paracrine function of endothelin-1. Such a theory is supported by the low concentration of endothelin-1 in the plasma, which is probably insufficient to activate endothelin receptors. Concentrations of angiotensin II and atrial natriuretic peptide in plasma are normally up to ten times greater than those of circulating endothelin-1. In addition, endothelin-1 has a half-life of less than 5 min in plasma, with the main clearance in the lungs and kidneys [17, 18]. It is likely that much higher concentrations of endothelin-1 occur at the junctions between endothelial and vascular smooth muscle cells and that at least some of the plasma endothelin-1 represents overspill from this site. One might conclude, therefore, that plasma levels of endothelin-1 in pathological states represent an unreliable index of vascular endothelin activity [19]. Conversely, urinary concentrations of endothelin-1 may reflect local renal endothelin activity, but not systemic endothelin function.

Endothelin receptors

Another important discovery is the identification of two seven-transmembrane G protein-coupled endothelin receptors, endothelin$_A$ and endothelin$_B$ receptor (ET_A and ET_B, respectively) [20, 21]. The isoforms of endothelin exert their physiological effects in a receptor-mediated fashion. The two subtypes of endothelin receptors can be distinguished pharmacologically by the order of their affinity for the three endothelin isopeptides; ET_A receptor is ET-1-selective, with an affinity order of ET-1 ≥ET-2 > ET-3, whereas ET_B receptor exhibits similar affinities for all three isopeptides [20, 21]. These receptors are both distributed in various tissues and cells, but with different levels of expression, suggesting the presence of a multifunctional ET system. ET_A receptors are located on vascular smooth muscle cells [20], and when activated, produce a sustained vasoconstriction that has a slow onset. In contrast, ET_B receptors are located on both endothelial [22] and vascular smooth muscle cells [23]. Activation of ET_B receptors on endothelial cells causes vasodilation

through the release of vasodilators acting on smooth muscle cells [24]. Moreover, ET_B receptor inhibits cell growth and vasoconstriction in the vascular system and functions as a clearance receptor, based on the evidence that selective ET_B receptor blockade inhibits the accumulation of intravenously administered radiolabeled ET-1 in tissue [3, 25]. The ET_B receptor-mediated clearance mechanism is particularly important in the lung, which clears about 80% of circulating ET-1 [26]. The ET_A receptor couples to the pertussis toxin insensitive $G\alpha_{q/11}$ to cause activation of phospholipase C, leading to an increase in inositol phosphate production and activation of protein kinase C in vascular smooth muscle cells [27, 28]. Judging from the data that activation of the ET_A receptor increases in cAMP production and protein kinase A activity [29–31], the ET_A receptor couples to $G\alpha_s$, the G protein that activates adenylate cyclase. Moreover, the ET_A receptor couples to $G\alpha_{12}$ and induces the stress fiber formation [31, 32]. On the other hand, the ET_B receptor couples to $G\alpha_{i/o}$ to inhibit cAMP formation [33–35], $G\alpha_{q/11}$ to stimulate phosphoinositide hydrolysis [36, 37], and $G\alpha_{13}$ to induce stress fiber formation [32, 38].

Pathophysiology of ET in pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a progressive and fatal condition characterized by a sustained increase in pulmonary vascular resistance, leading to right ventricular failure and premature death. ET-1 has been implicated as a mediator of increased vascular tone and vascular remodeling in pulmonary hypertension [39]. There is increasing evidence that pulmonary vascular smooth muscle cells, as well as endothelial cells, synthesize and release ET-1, particularly when stimulated by cytokines [40]. ET-1 is also produced in the lung in response to increased pressure. Expression of ET-1 mRNA increases in pulmonary vascular endothelial cells of patients with pulmonary hypertension [39]. In patients with pulmonary hypertension, a significant correlation between serum levels of ET and pulmonary vascular resistance, right atrial pressure, and oxygen saturation has been reported [41, 42]. In thromboembolic pulmonary hypertension, it was shown that there is upregulation of the ET_B receptor in the pulmonary artery [43]. Overexpression of ET-1 in experimental PAH models is localized to the medial layer of the pulmonary arterial tree [44]. ET_A and ET_B receptors are also upregulated [44]. Importantly, both nonselective (bosentan) and selective (sitaxentan, atrasentan, and TBC-3711) ET_A receptor antagonists are effective in PAH, reducing pulmonary artery pressure and inhibiting vascular remodeling in animal model studies. Bosentan was granted FDA approval for treatment of patients with NYHA/WHO functional class III or IV symptoms based on the results of two trials [45, 46]. Open-label continuation studies in each trial population demonstrated that the effects of bosentan were maintained beyond the initial 12-week study period. Survival at 1 and 2 years in the patients initially treated with bosentan was 96 and 89%, respectively, compared to predicted rates in historical controls of 69 and 57%. Complementary outcomes were obtained with sitaxentan [47]. Sitaxentan was granted a license for use in PAH in 2006. ET receptor antagonists are now established in American and European guidelines for the treatment of NYHA class III or IV patients with idiopathic PAH who either do not respond to acute vasodilators or remain class III despite vasodilator responsiveness.

Pathophysiology of ET in heart failure

Circulating ET-1 levels have been correlated with the severity of hemodynamics and with symptoms in patients with congestive heart failure [48, 49]. ET-1 contributes to acute and chronic increases in vascular resistance, ventricular and vascular remodeling, inflammation and arrhythmogenesis in models of heart failure. Tissue endothelin levels are increased in the failing human heart. Studies have also shown that big endothelin can be used as an independent predictor of survival [50]. It is likely that there is interplay between the ET system and neurohormonal system, because the activation of one system appears to increase

levels of the other. ET-1 appears to exert differential effects on the normal and failing myocardium. Patients with reduced left ventricular function have increased contractility in response to ET_A receptor blockade, whereas patients with normal left ventricular function manifest a reduction in contractility. ET_A receptors are upregulated in heart failure, whereas the ET_B receptor appears to be downregulated [51, 52]. Preliminary clinical trials with bosentan, darusentan, and BQ-123 show short-term hemodynamic benefits [53–55]. In addition, studies of intravenous tezosentan for patients with acutely decompensated heart failure were reported to improve cardiac index and pulmonary capillary wedge pressures [56]. However, five Randomized Intravenous TeZosentan (RITZ) trials for the treatment of congestive cardiac heart failure have been disappointing [57–63]. In stable patients with chronic heart failure, clinical trials of endothelin receptor antagonism also failed to demonstrate any benefit in clinically relevant end points (clinical status, mortality or hospitalization for heart failure) and were beset with toxicity problems [64, 65].

Pathophysiology of ET in systemic hypertension

The identification of endothelin as a vasoconstrictor [2] and the discovery of its release from vascular endothelial cells suggested that ET was involved in the pathogenesis of hypertension and vascular disease [66]. Further support for this hypothesis came from case reports of hemangioendothelioma patients who presented with markedly elevated levels of plasma ET-1 and hypertension, but who showed normalization of elevated ET-1 and blood pressure levels after tumor surgery [67]. In contrast, ET-1 plasma levels are generally normal in patients with essential hypertension; however, local ET-1 levels increase in the vascular wall with hypertension [68, 69]. Some studies demonstrate that the potent antihypertensive effects and end-organ protection by endothelin receptor antagonists in experimental hypertension are more effective in patients with high salt intake or angiotensin II level [70]. In addition, acute blockade of ET_A receptors ameliorates myocardial ischemia and biochemical changes caused by infarction in mice with coronary atherosclerosis [71]. Indeed, ET has strong growth-promoting activity in the vascular wall, and both endothelin and its receptors are widely expressed in macrophages, vascular smooth muscle cells, and fibroblasts [72]. Although plasma levels of ET are not consistently elevated in patients with systemic hypertension, there is often an exaggerated vasodilator response to ET receptor blockade in these patients [73]. This could contribute to a change in the sensitivity of the vasculature to endogenous ET-1 being altered as part of the disease. Other studies suggest that certain polymorphisms of the genes coding for ET-1 and endothelin receptors could be associated with chronic elevations in blood pressure [74]. In experimental animals with induced hypertension, ET_A receptor blockade prevents vascular hypertrophy and attenuates left ventricular hypertrophy [75]. Hypertension develops in ET_B knockout mice and blood pressure rises after ET_B blockade in humans [76, 77]. In patients with essential hypertension, the nonselective ET receptor antagonist TAK-044 caused greater forearm vasodilatation compared with normotensive controls, and the nonselective antagonist bosentan resulted in greater forearm vasodilatation than the selective ET_A receptor blocker BQ-123 [78, 79]. A 4-week treatment trial with bosentan, at a fairly high dose of 1,000 mg twice per day, produced a fall in ambulatory diastolic blood pressure of approximately 10 mmHg, an effect similar to treatment with 20 mg of enalapril [80]. These data suggest that ET antagonists may represent a new class of drug in the treatment of patients with uncontrolled hypertension. Moreover, ET_A receptor antagonists can reduce blood pressure substantially in hypertensive patients with chronic kidney disease [81]. This effect is synergistic to angiotensin-converting enzyme inhibitors and abolished by significant concurrent ET_B receptor blockade [82]. Furthermore, in diabetic and nondiabetic renal disease patients, ET receptor antagonists may produce favorable renal hemodynamic changes that reduce proteinuria [83, 84]. Thus, the ET antagonist may offer benefits to patients with chronic kidney disease that extend beyond blood pressure lowering.

Pathophysiology of ET in ovarian cancer

In addition to its role as a vasoconstrictor, ET-1 is known to be a potent mitogen that stimulates proto-oncogene expression in vascular and non-vascular cells. Elevated expression of ET-1 has been reported in many tumors, and it is believed to be a vital "hormone" in the growth and progression of prostate, ovarian, colorectal, bladder, breast, and lung carcinomas. Currently, the endothelin system in cancer biology has been an intense focus in both basic and clinical science settings. In particular, ET receptor activation plays a huge role in cancer cells or cancer-related cells, including proliferation, resistance to apoptosis, angiogenesis, migration, neovascularization, and subsequent invasion [85].

The ET-1 and ET_A receptor mRNA levels are detected in almost all primary and metastatic ovarian carcinomas. Their mRNA levels are especially higher in tumors than in normal ovarian tissues. A high level of ET-1 is found in ascitic fluids of ovarian cancer patients. In addition, ET_A expression is higher in grade 3 and 4 cancers than early grade ovarian cancers correlated. The high expression of ET-1 and its receptors in human cancer cells and human tumors further suggests a potential role for ET-1 in tumor growth promotion or maintenance through a possible autocrine or paracrine mechanism [86–90]. More importantly, ET_A is one of the genes more highly expressed in post-chemotherapy samples than in samples of untreated primary ovarian tumors [91].

As a growth-regulatory peptide, ET-1 also acts synergistically with other growth factors that have been implicated in cancer progression [92]. In particular, high ET-1 level is correlated with an increased vascular endothelial growth factor (VEGF), which is associated with neovascularization [90]. Transactivation of the epidermal growth factor (EGF) receptor in ET-1-induced mitogenic signaling in human ovarian carcinoma cells has been reported [93]. Furthermore, ET-1-induced cyclooxygenase-2 and prostaglandin E2 release and estrogen signaling have been shown to be important in the overall cellular proliferation processes [94, 95].

Many potential targets for the endothelin system have been suggested in ovarian carcinoma cells. One of the remedies includes the anti-tumor effect of green tea polyphenol epigallocatechin-3-gallate [96]. Two most actively studied drugs, however, are atrasentan (ABT-627, Abbott Laboratories, Abbott Park, IL) and zibotentan (ZD4054, AstraZeneca, Macclesfield, UK). These two pharmacological agents have oral bioavailability and bind to ET-1 receptor blocking signal transduction pathways implicated in cell proliferation and processes involving cancer growth [97–99].

Both atrasentan and zibotentan prevent ET-1-mediated survival signaling pathways and decreased proliferation in ovarian OVCA 433 and HEY cells. These agents significantly reduce tumor growth in animals bearing ovarian tumor xenografts in vivo. Furthermore, many clinical studies have shown that blocking ET-1 receptor inhibits tumor growth and/or reduces metastatic potential of ovarian cancer [100–104]. Treatment with either agent provides no detectable signs of acute or delayed toxicity. Both atrasentan and zibotentan give comparable results to those of paclitaxel. However, a combinational therapy of ET-receptor blocker and paclitaxel provide a better recovery or prolonged tumor growth inhibition. It is believed that atrasentan or zibotentan can actually enhance paclitaxel activity in human ovarian carcinoma in vitro and in vivo [89, 102, 104].

Conclusions and perspective

Since its discovery over 20 years ago, endothelin has been recognized to function not only as a vasoconstrictor but also as a multifunctional peptide with cytokine- or hormone-like activity. From the basic science perspective, ET has been shown to regulate a wide spectrum

of physiological cellular activities, including mitogenesis, cell survival, angiogenesis, bone remodeling, stimulation of nociceptors, tumor-infiltrating immune cells, epithelial-to-mesenchymal transition, invasion, and metastatic dissemination. From the clinical perspective, the therapeutic efficacy of ET antagonists has become the first clinical indication for pulmonary arterial hypertension. Other cardiovascular-related diseases have shown promising clinical indications. Furthermore, exciting new results from basic science studies have associated endothelin antagonist therapy to many other cancer-related diseases, including targets for pharmacotherapy of female malignancies [105]. Without doubt, a detailed understanding of the molecular mechanisms of endothelin is a crucial step in identifying new effective therapies for other diseases.

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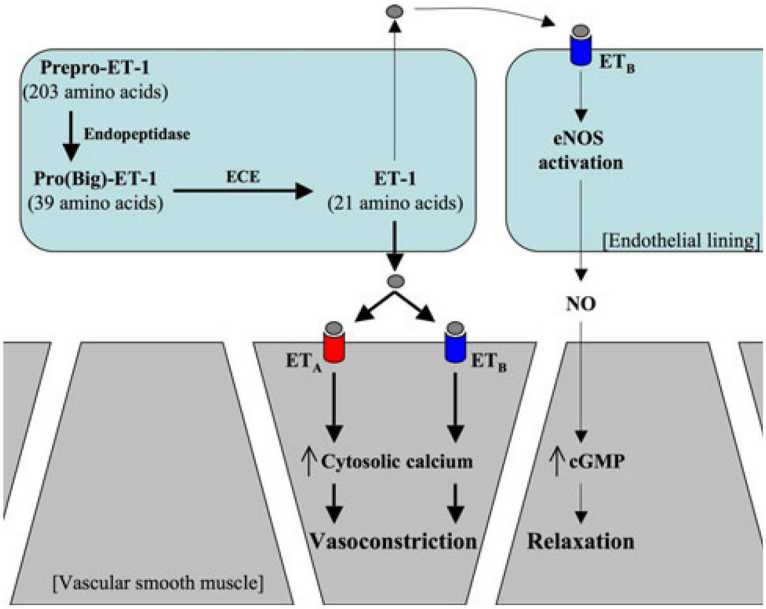

Fig. 1.

Endothelin-1 (ET-1) is transcripted and translated as a prepro-ET-1. Dibasic-pair-specific endopeptidase cleaves prepro-ET-1 to form pro-ET-1 or big ET-1. The precursor big ET-1 is further cleaved by endothelin-converting enzyme (ECE) to the vaso-active peptide ET-1. ET-1 can activate endothelin receptors type-A (ET_A) and type-B (ET_B). While ET_A is localized in vascular smooth muscle cells, ET_B resides in vascular smooth muscle and endothelial cells. Activation of ET_A or ET_B in smooth muscle cells results in vasoconstriction. Activation of ET_B in endothelial cells will induce nitric oxide (NO) production through endothelial nitric oxide synthase (eNOS). NO is a potent vasodilator in smooth muscle cells. Activation of ET receptors in non-vascular cells has also been implicated with other cellular functions (see text for more details)