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Review Article

A REVIEW ON ENDOTHELINS: AN UPDATE

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ABSTRACT

Endothelin (ET) is the most potent vasoconstrictor. It is secreted by the endothelial cells. At low concentration, it acts as an agonist for endothelium-derived relaxing factors and thereby causes vasodilatation, and at higher concentration it acts as a potent vasoconstrictor. It is synthesized by proteolytic cleavage of preproendothelin to proendothelin by the action of metallopeptidases and chymase, which is further cleaved into mature form of ET by endothelin converting enzyme. There are four isoforms of ET, namely, ET-1, ET-2, ET-3, and ET-4. ET acts on 2 types of receptors. Binding of ET-1 to ET_A receptor at the vascular smooth muscle cells induces vasoconstriction. It also produces vasoconstriction by acting on the ET_{B2} receptor of vascular smooth muscle cells but promotes vasodilatation at ET_{B1} receptor present on the endothelial cell.

Keywords: Endothelin, Vasoconstriction, Endothelin receptors, Endothelin agonists, Antagonists.

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INTRODUCTION

Vascular endothelium is the monolayer of epithelial cells which lines the entire circulatory system [1,2]. They are situated on the tunica intima which forms the innermost lining of the vasculature [3]. It is widely recognized that endothelial cells exhibit heterogeneity that they differ in their properties at different anatomical sites [4]. Arteries, veins, and capillaries exhibit different expression of endothelial layer. In arteries and veins endothelium is surrounded by thick single or multiple layers of muscle cell along with elastic fibers, while capillaries are composed entirely of endothelium and a basement membrane to support it [5].

The endothelial cells trigger the production and secretion of endothelium-derived relaxing and constricting factors directly into the bloodstream which exhibit their actions on the adjacent cells protecting the vessels [6]. Endothelium has proven to be a highly specialized endocrine organ involved in performing a wide variety of significant functions required to maintain hemostasis [7].

Endothelin (ET)

ET is a vasoactive peptide secreted by the endothelial cells. It acts as an agonist for endothelium-derived relaxing factor (EDRF) at low concentrations. However, at higher concentration acts it as a vasoconstrictor [8]. It is the most potent vasoconstrictor discovered till date and is 10 times more potent than angiotensin II who previously was the record holder [9]. It shows incredible resemblance to the snake venom sarafotoxins (SRTXs), belonging to atractaspis genus which shares the same receptor [10]. The primary function of ET is vasoconstriction [11].

History

The concept of release of vasoactive factors emerged succeeding the discovery of EDRF by Furchgott and Zawadzki in the year 1980 [12]. In 1987, the endogenous substance was identified as nitric oxide [13]. In 1989, three isoforms of ET were isolated, namely, ET-1 ET-2 and ET-3 [14]. Subsequently, vasoactive intestinal factor or ET-4 was discovered through analysis of mouse genome [15]. In the year 1990, two ET receptors (ET_A and ET_B) were successfully cloned, and ET_A receptor antagonist (BQ 123) was developed in 1992. Bosentan, ET antagonist, was developed 1993. Bosentan was approved by the Food and Drug Administration (FDA) in the year 2001 for the treatment of pulmonary arterial hypertension. During 2007–2013 ambrisentan and macitentan gained approval for the treatment of pulmonary artery hypertension, respectively [16].

BIOSYNTHESIS OF ET

Preproendothelin mRNA transcription

The production of ET is regulated at gene level [17]. The EDN1 gene codes for inactive precursor polypeptide preproendothelin-1 comprising 212 amino acid residues [18]. EDN2 and EDN3 genes code for preproendothelin 2 and preproendothelin 3, respectively [19].

Proteolytic cleavages and release of active form

Preproendothelin is cleaved to generate a 38-amino acid intermediate peptide pre-ET (big ET) by specific furin-like proteases, which is further cleaved by specific metallopeptidase called endothelin converting enzymes (ECE), non ECE metallopeptidases, endopeptidases, and chymase [20] into 21 amino acid bioactive peptide ET. The steps involved in the production of ET-4 are as follows.

Prepro vasoactive intestinal contractor mRNA transcription

The prepro vasoactive intestinal contractor gene codes for mRNA which is then translated to precursor protein.

Proteolytic cleavage to release the active form

The precursor protein formed is cleaved to big vasoactive intestinal contractor by the action of endopeptidases. It is then further cleaved to form mature vasoactive intestinal contractor by converting enzymes.

Isoforms of ETs

ET constitutes a family of four endogenous isopeptides, namely, ET-1, ET-2, ET-3, and ET-4.

ET-1

It is encoded by gene EDN1. It chiefly involved in cardiovascular actions and is potent endogenous vasoconstrictor. Its plasma concentration is much less which is inadequate to activate the ET receptors. The concentration within the vascular wall is greater than the circulating concentration because its secretion mainly occurs on the basal side of the endothelial cells [21].

ET-2

It is encoded by gene EDN2. It differs from ET-1 in two amino acid residues. It is less widely distributed and has been identified in

endothelial cells, heart, lungs stomach, and intestine, to less extent in myocardium, placenta, and uterus [22]. Recent studies have shown its presence in ovaries, and ET-2 mediated contractions suggested a final indicator facilitating ovulation contributing its significance in ovarian physiology [23].

ET-3

ET-3 is coded by EDN3 gene. Its structure contains six different amino acid residues than that in ET-1. It is present in lung, gastric intestinal tract, kidney, and adrenal glands. In intestine and brain, it is expressed in higher concentrations.

ET-4

It differs in only one amino acid from ET-2. Southern blot analysis, cloning and sequencing have shown that it is mouse and rat counterpart of the human ET-2.

Factors affecting biosynthesis of ET

Under pathophysiological conditions, several cells such as endothelial cells, vascular smooth muscle cells, inflammatory cells, cardiomyocytes, leukocytes, and mesangial cells produce ET-1. Its expression is stimulated by ET itself and also by many other factors such as insulin, growth factors, catecholamine, angiotensin II, glucose, and cholesterol. The inhibitors of ET synthesis are prostacyclin, estrogen, nitric oxide, heparin, natriuretic peptides, and prostaglandins.

ECE

A metallopeptidase inhibitor phosphoramidon inhibited the generation of ET-1 from big ET. The protease was identified and named as ECE-1. ECE-1 is a zinc type II integral membrane metallopeptidase protein [24]. Four isoforms have been successfully identified, namely, ECE-1a, ECE-1b, ECE-1c, and ECE-1d. These are encoded by a single gene but under the influence of different promoter [25]. ECE-1a is present in intracellular secretory vesicles which are then transferred to the cell surface while ECE-1b is present intracellularly close to trans-Golgi networks. ECE-1c and ECE-1d are predominant on the cell surface and may function as ectoenzymes. ECE proteolytically cleaves big ET to its active form ET. Furthermore, it is also involved in the hydrolysis of different peptides such as bradykinin, substance P, and insulin.

ECE inhibitors

Phosphoramidon is an inhibitor of both ECE-1 and ECE-2 [26]. Over the years several inhibitors of ECE were discovered, namely, CGS35066 and its prodrug CGS35339. SLV306 inhibits both ECE and neural endopeptidase enzyme (NEP). SCH54470 inhibits angiotensin-converting enzyme (ACE) along with ECE and NEP [27].

ET receptors

Studies have proven ET acts on three different receptor subtypes, namely, ET_A, ET_B, and ET_C. ET receptor subtypes belong to G-protein coupled receptor family and ranges in size from 45,000 to 50,000 Daltons in different tissues.

ET_A receptor

The gene EDNRA codes for ET_A receptor. It is composed of 427 amino acid residues. Its proportion is greater in vascular smooth muscles and peripheral tissues [28]. Order of potency is ET-1> ET-2>>ET-3 [29]. Studies have also suggested the presence of different forms, i.e. ET_{A1} and ET_{A2} receptors that are BQ-123 sensitive and insensitive, respectively [30].

ET_B receptor

It comprises 442 amino acid residues and is coded by EDNRB gene. They are localized on endothelial cells and to less extent in vascular smooth muscles, fibroblasts, and macrophages [31]. Lungs and liver are richly expressed, and kidney shows unusually high density [32]. It can be distinguished into ET_{B1} which are present on the endothelial cells and ET_{B2} those present on the vascular smooth muscle. ET_A and ET_B show 64% amino acid sequence similarity.

ET_C receptor

It shows specific affinity toward SRTX 6c and ET-3 and its presence is marked in cells of different species and yet to be characterized for human tissues. Five ET receptors have been cloned so far. ET_A and ET_B receptors in mammals, a dual angiotensin II/ET-1 receptor in rats, a novel ET_B receptor in birds and ET-3 selective ET_C receptor in frogs.

Transducer mechanism

The binding of ET-1 to ET_A receptor activates phospholipase C leading to hydrolysis of phosphatidylinositol to inositol 1, 4, 5-triphosphate (IP_3), and diacylglycerol (DAG). IP_3 transiently increases the concentration of intracellular calcium (Ca^{2+}) by its mobilization from sarcoplasmic reticulum. A sustained increase is observed when Ca^{2+} is flooded through store-operated Ca^{2+} channels by the action of IP3. The receptor-operated channels are opened through DAG which promotes the Ca^{2+} influx through the activation of various ion channels resulting in vasoconstriction. Increased intracellular Ca^{2+} concentration may open Ca^{2+} activated chloride channels that cause chloride efflux and depolarizes the cell leading to activation of voltage-dependent Ca^{2+} channels causing Ca^{2+} influx. ET-1 inhibits the voltage-dependent potassium channels present in pulmonary artery vascular smooth muscle cells causing depolarization and influx of Ca^{2+} through voltage-dependent Ca^{2+} channels [33]. Intracellular Ca^{2+} may also be involved in the activation of Na^+/H^+ exchanger following alkalization of cells and promoting intracellular flooding of Ca^{2+} through Na^+/Ca^{++} exchanger. Intracellular Ca^{2+} boosts release of Ca^{2+} through intracellular stores through ryanodine receptors causing smooth muscle to engage in constriction. A negative feedback mechanism is generated to diminish Ca^{2+} signaling by DAG and Ca^{2+} activated protein kinase C.

ET_{B2} receptor situated on the vascular smooth muscle cells mediates vasoconstriction with a mechanism like ET_A subtype. ET-1 causes vasodilation with a cascade of events that begins with activation of phospholipase A2 by binding to ET_{B1} receptor confined to endothelial cell surfaces causing release of arachidonic metabolites. The cAMP, thus, stimulated decreases the levels of intracellular Ca^{2+} ions causing vasodilatation [34]. Activation of ET_{B1} receptors also causes the release of nitric oxide due to stimulation of endothelial nitric oxide synthase (eNOS). Fig. 1 shows the mechanism of ET.

PHARMACOLOGICAL ACTIONS OF ENDOTHELINS

ET-1 action on ET_A receptor induces mitogenesis, promotes the growth and proliferation of different cells such as fibroblasts, endothelial cells, astrocytes, and smooth muscle cells. It exhibits positive inotropic and chronotropic effects, vasoconstriction and control of water, and sodium retention. It intensifies the vasoconstrictive action of norepinephrine through ET_A receptors, triggers the production of atrial natriuretic peptide and nitric oxide by heart and vascular endothelial cells, respectively. It induces hypertrophy in different tissue including cardiomyocytes, cardiac fibroblasts, astrocytes, renal interstitial fibroblasts, mesangial cells, and vascular smooth muscle cells. It causes inflammation by increasing vascular permeability, activating mast cells, and promoting cellular adhesion. They are also associated in embryonic development, bronchoconstriction, prostrate growth, carcinogenesis, gastrointestinal functions, and endocrine functions. Its action on ET_B receptor inhibits ECE-1 expression in the endothelial cells and prevents apoptosis. Natriuresis and diuresis occur by the action of ET-1 on ET_A and ET_B receptors.

Clearance of ET

Clearance of ET-1 is very rapid and generally occurs in kidney and lungs [35], and its half-life is <5 min [36]. Pulmonary circulation clears approximately 50% of circulating ET-1 mediated through ET_{B1} receptors by inducing their internalization and degradation.

ET agonists

ET-1 is mainly used during clinical studies in volunteers. IRL1620 was initially developed as an agonist for ET_B receptor, but now it is being used in clinical trials as a vasodilator.

ET antagonists

The first ET receptor antagonist BQ123 was published 2 years after the cloning of ET receptors. Many peptide and non-peptide antagonists are now available, and some are undergoing clinical development. Since majority of them are peptides, they need intra-atrial administration. They get hydrolyzed by the action of peptidases in the systemic circulation and gastrointestinal tract. Therefore, non-peptide antagonists have been developed with improved bioavailability which is potent. At present, only two diseases have been approved for ET receptor antagonist, which are pulmonary artery hypertension and scleroderma-related digital ulcers [37-39]. Table 1 shows the properties, agonists and antagonists of ET receptors.

ROLE OF ET IN CARDIOVASCULAR DISORDERS

ET is being studied as a novel target in the therapy of various cardiovascular disorders.

Hypertension

Increase in the concentration of ET-1 causes a subsequent increase in total peripheral resistance which may contribute to the development of hypertension. In a clinical study, treatment with darusentan an ET_A receptor antagonist to patients with elevated systolic blood pressure (>140 mm Hg) showed a notable lowering of blood pressure compared to placebo group, in all cases included a diuretic and a drug acting on the renin-angiotensin system.

Pulmonary hypertension

The vasoconstrictive properties of ET_B receptors may become prominent in this disease. The concentration of ET-1 and ET_A receptors are also increased in lungs. The vasoconstrictive property of the ET_B may prove advantageous in the use of dual antagonists over receptor ET_A blockade [40]. Till date ambrisentan, bosentan and macitentan have been clinically approved by the FDA. Sitaxentan exhibits higher ET_A selectivity was successfully evaluated for its treatment and had received approval in Europe in the year 2007 but was withdrawn due to fatal cases of hepatic failure as a side effect in 2010.

Heart failure

Plasma concentrations of ET-1 are higher in patients with heart failure resulting from increased production or decreased clearance of the peptide. In myocardium, ET-1 produces concentration-dependent inotropic effects through ET_A receptors. Its contribution to pathophysiology may be due to its paracrine action and as a circulating hormone leading to vasoconstrictor effects. ET_A selective inhibition and ECE-1 inhibition by phosphoramidon significantly produced vasodilatation in patients with heart failure. Adverse consequences were seen by blocking of ET_B receptors that pointed out the receptor function complications. In a clinical study conducted with Bosentan when administered to patients with severe heart failure resulted in decreased vascular and pulmonary resistances, atrial pressure and increase in cardiac output. As a side effect, heart failure has been reported with the drug avosentan, and the risk of developing heart failure was increased with Atrasentan in a study conducted for its use in the treatment for prostate cancer.

Atherosclerosis

A disturbance in the hemostatic balance between vasoconstrictors and vasodilators and hypercholesterolemia leads to endothelial dysfunction, causing impairment of endothelium-dependent vasodilation and damage to the arterial wall. Oxidized low-density lipoprotein levels promote the production of ET-1. The concentration of ET-1 increases in plasma and tissues which triggers the synthesis of transforming growth factor β1, basic fibroblast growth factor, epiregulin, platelet-derived growth factor, and different molecules involved in atherogenesis. It also induces neutrophil and platelet adhesion stimulating lesion growth and coronary thrombosis. This indicates the role of ET-1 for the progression of atherosclerosis by boosting lipid biosynthesis. Altered ET receptors expression was also reported in atherosclerotic patients with more number of ET_B receptors in atherosclerotic arteries that were confined to inflammatory cells and vascular smooth muscles. It was also proposed that switching between ET_A and ET_B receptors by foamy macrophages and T lymphocytes could be important criteria for the advancement of atherosclerosis. Blocking of ET receptor results in antiatherogenic action.

ROLE OF ENDOTHELIN IN CHRONIC KIDNEY DISEASE

ET-1 is also expressed in renal tubular epithelial cells especially of the medullary collecting duct. Both the receptors ET_A and ET_B are present in renal vascular smooth muscle, but ET_B dominates tubular epithelial cells. Under normal physiological conditions, it causes vasoconstriction by binding on ET_A receptors present on renal smooth muscle. In disease conditions where the expression is increased it causes proliferation of mesangial cells, vascular remodeling and development of renal fibrosis. It also inhibits salt and water reabsorption causing diuresis and natriuresis, increases glomerular permeability to albumin and induces

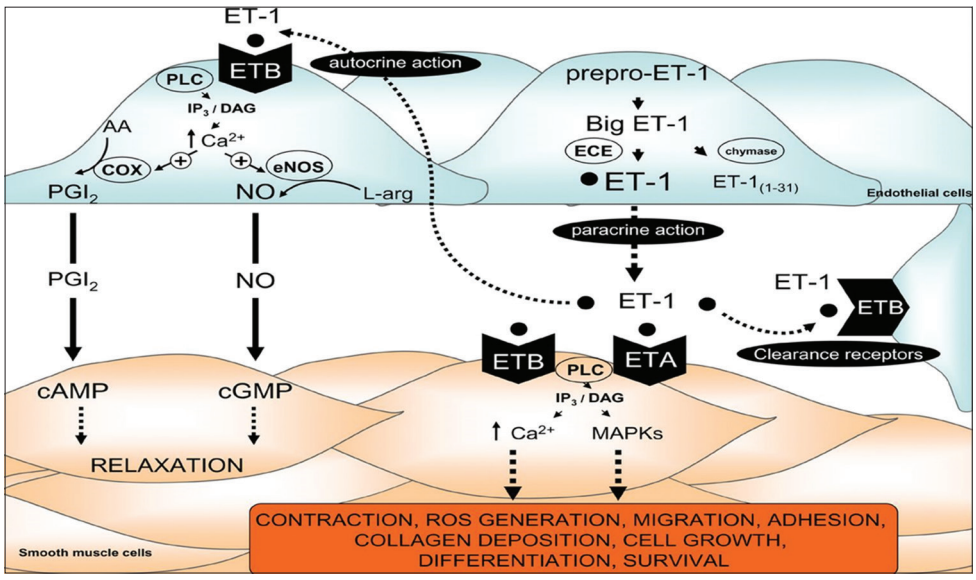

Fig. 1: Mechanism of action of endothelin

Table 1: Properties, agonist and antagonists of ET receptors

S. No.	Feature	Receptors	
		ET_A	ET_B
1	Location	Vascular smooth muscles, myocytes, organs, and peripheral tissues	Endothelial cells, vascular smooth muscles, organs such as kidneys, lungs, and liver
2	Nature	G-protein coupled receptor	G-protein coupled receptor
3	Transducer mechanism	Gs and $G_{12/13}G_{q/11}$ PLC - IP_3/DAG↑, cystolic Ca^{+2}↑	G_i and $G_{q/11}$ PLC-IP_3/DAG↑Cystolic Ca^{+2}↑, MAPK (contraction) activates PLA_2, NO synthase (dilatation)
4	Actions	Vasoconstriction, smooth muscle contraction, cell proliferation, vascular growth, and remodeling	(ET_{B2}) vasoconstriction and (ET_{B1}) vasodilatation, smooth muscle relaxation, antiproliferation, renal blood pressure regulation
5	Agonist	ET-1, ET-2, ET-3	ET-1, ET-2, ET-3 SRTX 6c, BQ3020
6	Antagonists	A-127772, A-182086 ABT-627, ambrisentan, atrasentan, BE-18572A/B BMS-182874, BMS-193884, bosentan, BQ-123, BQ-153, BQ-162, BQ-485, BQ-610, CGS-27830, clazosentan, darusentan, EMD-122946, EMD-92426, edonentan, enrasentan, FR-139317, IRL-3630, J-104121, J-104132, L-744453, L-749329, LU127043 LU302146, PD-14795, PD-151242, RO46-2005 RO 48-5695, S-0139, SB-209670, sitaxentan, TA-0115 TA-0201, TAK-044, tezosentan WS-7338B, ZD 1611, zibotentan	A182086 CGS-27830 Enrasentan IRL-3630 L-753037 LU224332 LU302872 PD-142893 PD-145065 RO46-2005 RO48-5695 SB-209670 TA-0201 Nonselective: Bosentan, macitentan, TAK-044

ET: Endothelin, SRTX: Sarafotoxins

renal inflammation. Studies have shown that high salt intake promotes its production and release. Chronic kidney disease is characterized by elevated levels of ET-1. Studies have proven that the blockade of ET_A receptors has improved kidney functioning in a diabetic model of rats, with effects far better than ACE inhibitors. With positive results from preclinical studies, now clinical trials conducted with many ET receptor antagonists such as atrasentan, avosentan, darusentan, and sitaxentan shows reduced proteinuria in patients suffering from chronic kidney disease. Regardless, no ET antagonists have been approved for its treatment due to ongoing studies on their long-term adverse effects [41].

ET AS THERAPEUTIC TARGET IN CANCER

ET-1 is produced by different cells including cancer cell. It helps the cells to proliferate, aids in angiogenesis, prevents them from dying and inhibits apoptosis [42]. It is also suspected to exhibit a concealed role in cancer pain. In the mid-1990's researchers detected the presence of more ET-1 in adenocarcinomas. Atrasentan, an ET receptor antagonist, was tested by Pittsburgh's Nelson in the year 2002 for the treatment of adenocarcinomas. In the succeeding year, they conducted a placebo-controlled trial with 288 patients which showed promising outcomes with the decrease in the progression of the disease. However, the side effects of these drugs hindered their way to the market [43].

Zibotentan (ET_A antagonist) has also been evaluated for its anticarcinogenic activity in patients suffering from castration-resistant prostate cancer [44]. In breast cancer, the elevated circulating levels of ET-1 were found which may contribute to its early prediction. ET_A receptors have shown its importance in cancer cell metastasis and lymphatic angiogenesis while ET_B receptors are responsible for inhibiting the migration of T cells to tumors. In ovarian cancer, ET stimulates the changeover of epithelial cells to mesenchymal cells. Zibotentan and Atrasentan have also shown encouraging results for the decrease in the progression of the disease in pre-clinical models for ovarian cancer. Further, clinical trials are required to demonstrate the therapeutic potential of ET receptor antagonists in the therapy of life-threatening cancer diseases.

CONCLUSION

ET can be used as a potential target in diseases that shows its involvement. ET receptor antagonists such as bosentan, ambrisentan, and macitentan have been approved by the FDA for the treatment of pulmonary artery hypertension. Its role has been proven in many pathophysiological conditions including cardiovascular diseases, chronic kidney disease, and cancer. Due to the wide variety of actions of ET on human vasculature, it can be regarded as a potential therapeutic target in the treatment of many life-threatening diseases. Many clinical trials are being conducted for its potential activity and efficacy for its use in these diseases with hope of developing a beneficial therapy to the patients.

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AUTHORS CONTRIBUTION

All authors equally contributed in the preparation of the manuscript.

CONFLICTS OF INTEREST

Authors report no conflict of interest.

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