*Critical Review***Pathophysiological Roles of Endothelin Receptors in Cardiovascular Diseases**Mamoru Ohkita¹, Masashi Tawa^{1,2}, Kento Kitada^{1,3}, and Yasuo Matsumura^{1,*}¹Laboratory of Pathological and Molecular Pharmacology, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan²Department of Pharmacology, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan³Department of Pharmacology, Kagawa University Medical School, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

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Abstract. Endothelin (ET)-1 derived from endothelial cells has a much more important role in cardiovascular system regulation than the ET-2 and ET-3 isoforms. Numerous lines of evidence indicate that ET-1 possesses a number of biological activities leading to cardiovascular diseases (CVD) including hypertension and atherosclerosis. Physiological and pathophysiological responses to ET-1 in various tissues are mediated by interactions with ET_A- and ET_B-receptor subtypes. Both subtypes on vascular smooth muscle cells mediate vasoconstriction, whereas the ET_B-receptor subtype on endothelial cells contributes to vasodilatation and ET-1 clearance. Although selective ET_A- or nonselective ET_A/ET_B-receptor antagonisms have been assumed as potential strategies for the treatment of several CVD based on clinical and animal experiments, it remains unclear which antagonisms are suitable for individuals with CVD because upregulation of the nitric oxide system via the ET_B receptor is responsible for vasoprotective effects such as vasodilatation and anti-cell proliferation. In this review, we have summarized the current understanding regarding the role of ET receptors, especially the ET_B receptor, in CVD.

Keywords: endothelin-1, endothelin receptor, endothelial cell, vascular smooth muscle cell, cardiovascular disease

1. Introduction

Endothelin (ET) derived from vascular endothelial cells (ECs), which consists of a 21 amino acid peptide, has a strong and persistent vasoconstrictive action (1). ET has three family peptides (ET-1, ET-2, and ET-3). As the distribution and properties of these peptides are different, each peptide is believed to play specific physiological roles. ET has two types of receptor: the ET_A receptor with a high affinity for ET-1 and ET-2 is mainly located on muscle cells, whereas the ET_B receptor with an affinity for all three peptides lies on endothelial, epithelial, endocrine, and nerve cells. Of the three ET iso-

forms, ET-1 plays a much more important role in the regulation of vascular tone than the others and has a powerful effect on the cardiovascular system. Thus, the role of ET-1 and its receptors as the etiology or precipitating factors in various cardiovascular diseases (CVD) has been investigated (2, 3). In addition, numerous studies have reported effective treatment targeted at ET-1 in pulmonary hypertension, salt-sensitive hypertension, diabetes, and acute and chronic kidney diseases using ET-converting enzyme (ECE) inhibitors and ET-receptor antagonists (2, 4). Several animal models genetically lacking ET-1 and ET receptors have also been used as a tool for determining the physiological and pathophysiological roles of ET-1 and ET receptors in CVD (5–10).

2. ET system

ECs are known as the main physiological source of vascular ET-1. Vascular smooth muscle cells (VSMCs),

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macrophages, leukocytes, cardiomyocytes, and fibroblasts are also capable of ET-1 production (11–13). Several studies have indicated that various physical and chemical factors such as thrombin, angiotensin II, cytokines, hypoxia, and shear stress stimulate ET-1 gene expression in ECs by DNA binding of transcription factors including activator protein-1, GATA-2, Smad, nuclear factor-kappa B (NF- κ B), and hypoxia inducible factor-1 (14–18). On the other hand, ET-1 is synthesized as an inactive 203-amino-acid precursor, preproET-1, which is proteolytically cleaved to yield a second inactive 39 (or 38)-amino-acid segment called ‘big’ ET-1. The last part of the proteolytic process is mainly carried out by ECE (ECE-1 and ECE-2) and leads to the production of the bioactive form of 21-amino-acid peptide ET-1. As ET-1 release from ECs is constitutive, ET-1 biosynthesis and release appear to be mainly controlled via regulation of gene transcription and/or ECE activity.

The downstream effects of ET-1 are mediated by two G-protein-coupled receptors ET_A and ET_B. In the vasculature, the ET_A receptor on VSMCs mediates vasoconstriction and cell proliferation, whereas the endothelial ET_B receptor (generally called ET_{B1}) exerts opposite effects. Stimulation of the ET_{B1} receptor leads to the release of vasodilators such as nitric oxide (NO) and prostaglandin I₂ and clearance of ET-1 from the circulation within the lungs, kidneys, and liver (19–23). On the other hand, although another ET_B-receptor subtype (ET_{B2}) located on VSMCs exerts vasoconstriction, it has become clear that ET_{B2} receptor-induced vasoconstriction is negligible under normal conditions but becomes more important in some kinds of diseases such as atherosclerosis and essential hypertension (24–26).

Many vascular relaxing or contraction factors produced in the blood vessel wall maintain normal endothelial function by mutually antagonistic actions. In particular, there are various reports regarding the interaction of ET-1 and NO (27). For instance, ET-1 binding to the ET_{B1} receptor leads to phosphoinositide 3-kinase (PI3K) activation and subsequent production of phosphatidylinositol-3,4,5-trisphosphate, which results in recruitment and activation of protein kinase B / Akt (28). This PI3K/Akt pathway is responsible for the phosphorylation and activation of endothelial NO synthase (eNOS). On the other hand, ET-1 also reduces eNOS expression and its activity through increases in hydrogen peroxide by the ET_A receptor (29). Therefore, reduced ET_B-receptor function and/or overactivation of the ET_A receptor eliminate the protective function by NO in vessels and promote the pathological formation of various circulatory diseases (Fig. 1).

3. Pathophysiological roles of ET receptors

3.1. Cardiac disease

Myocardial infarction results from the formation of an atherosclerotic plaque and ultimately leads to heart failure. Several studies have reported elevations in plasma ET-1 levels in patients with coronary artery diseases such as angina (30), myocardial infarction (31), and immediately after percutaneous transluminal coronary angioplasty (32). In addition, the production and release of ET-1 as well as ET-receptor expression is enhanced during myocardial ischemia/reperfusion (33, 34). Thus, these findings suggest that endogenous ET-1 plays an important role in the pathophysiology of myocardial ischemia/reperfusion.

Locally generated ET-1 contributes to tissue repair or remodeling of the infarcted heart in an autocrine/paracrine manner, thereby exerting an immediate beneficial effect on damaged tissue (33, 34). Other studies reported that ET-1 administered prior to the onset of ischemia exhibited cardioprotective effects (35, 36). Exogenous ET-1 mimics the cardioprotective effect of pre-conditioning against infarction, apparently via ET_A receptor-mediated activation of PKC and a mitochondrial type of ATP-sensitive K⁺ channel (37, 38). On the other hand, a substantial and long lasting rise in ET-1 induces myocardial hypertrophy, which is associated with a maladaptive effect on myocardial structure and function, thereby leading to fatal events (39–42). The ET-1 / ET_A receptor pathway also promotes myocardial fibrosis by enhancing cardiac fibroblast proliferation, adhesion molecule expression, and extracellular matrix deposition (43, 44). Therefore, the use of ET-receptor antagonists, mostly targeting the ET_A receptor, provides beneficial effects in chronic heart failure as evidenced by a reduced infarct size, improved reperfusion coronary flow, or protection during ischemia/reperfusion (39–42).

In myocardial ischemia, sympathetic overactivity accompanied by excessive norepinephrine (NE) release is associated with cardiac dysfunction and arrhythmia, thereby exaggerating primary ischemia and initiating a malignant cycle that can cause further myocardial damage and high-risk cardiac dysfunction (45, 46). Both ET_A and ET_B receptors exist in the sympathetic nerve varicosities of guinea pig hearts and exhibit opposite effects on NE release in association with reperfusion arrhythmias: ET_A receptors evoke NE release, whereas ET_B receptors prevent it (47). Previous studies showed that a selective ET_A-receptor antagonist or the combination of ET_A- and ET_B-receptor antagonists suppressed excessive NE release from sympathetic nerve endings in post-ischemic rat hearts and improved cardiac dysfunction after ischemia/reperfusion (9, 47). In addition, exogenous

Fig. 1. Schematic illustration of ET-1 production and ET receptor-mediated actions on vascular endothelial cells and smooth muscle cells. G: G protein, ROS: reactive oxygen species, CaM: calmodulin, AA: arachidonic acid, PGI₂: prostaglandin I₂, AC: adenylate cyclase, sGC: soluble guanylate cyclase.

ET-1 induced excessive NE release and subsequent cardiac dysfunction, counteracted with the Na⁺/H⁺ exchanger (NHE) inhibitor 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA). Thus, the excessive NE overflow triggered by the ET-1 / ET_A / NHE pathway seems to be contributive to post-ischemic cardiac dysfunction in rats (Fig. 2). On the other hand, both pharmacological blockade and genetic deficiency of ET_B receptors exaggerated the post-ischemic excessive NE release and cardiac dysfunction. Oikonomidis et al. demonstrated that NE levels during the early phase of myocardial infarction are much higher in ET_B-deficient rats than wild-type rats and this contributes to the incidence of ventricular arrhythmogenesis, thereby suggesting that the ET_B receptor exerts a suppressive effect in sympathetic hyperactivity during the early phase of myocardial infarction (48). Furthermore, activation of the ET_B receptor with sarafotoxin-6c substantially reduced NE release, myocardial infarct size, and ischemic arrhythmias (47, 49). Taken together, acti-

vation of the ET_B receptor may likely prevent post-ischemic cardiac dysfunction through the attenuation of NE release.

Big ET-1 exerts several physiological actions similar to ET-1, but it must be converted to ET-1 via enzymatic degradation by ECE (50). ECE expression and its enzyme activities are increased in myocardial infarction (51), thereby suggesting that a selective ECE inhibitor may be useful in ischemic cardiac diseases at the clinical level, which warrants further attention. In fact, SM-19712 and FR901533, both of which are highly selective ECE inhibitors, exert a desirable influence on myocardial infarction by decreasing plasma concentrations of ET-1 (52, 53). On the other hand, exogenously applied big ET-1 has qualitatively similar effects to ET-1 in the cardiovascular system in vivo and in vitro (54 – 57). Against this background, Sharif et al. demonstrated that exogenously applied ET-1 exhibited opposite effects to endogenously released ET-1 on ischemic ventricular arrhythmias (58).

Fig. 2. Proposed explanation for the interaction between the ET-1 system and norepinephrine (NE) release from cardiac sympathetic nerve endings in protracted myocardial ischemia. ATP is depleted and axoplasmic pH is reduced under ischemic conditions. This diminishes vesicular storage of NE, leading to a large increase in free axoplasmic NE. Compensatory activation of the neuronal Na⁺/H⁺ exchanger (NHE) by axoplasmic acidification causes influx of Na⁺ in exchange for H⁺. The resulting Na⁺ accumulation triggers a massive release of free axoplasmic NE via a reversal of the NE transporter (NET). Released NE acts on postsynaptic adrenoceptors on myocytes. Stimulation of the ET_A receptor existing in sympathetic nerve endings by endogenously generated or exogenously applied ET-1 enhances neuronal NHE activity and results in increases in NE release. In contrast, exogenously applied big ET-1 is converted to ET-1 by ECE-1 expressed on the cell surface, and this ET-1 preferentially binds to the ET_B receptor located on NOS-containing cells. As a result, increments in NO production cause inhibition of NE release. NCX: Na⁺/Ca²⁺ exchanger, VMAT: vesicular monoamine transporter.

We also reported that exogenous big ET-1 suppressed ischemia/reperfusion-induced NE overflow and improved cardiac dysfunction observed after reperfusion, in spite of the fact that ET-1 content in coronary effluent from the heart exposed to ischemia/reperfusion was increased by exogenous big ET-1 application (59). In addition, treatment with big ET-1 in the presence of A-192621, a selective ET_B-receptor antagonist, failed to exert beneficial effects against ischemia/reperfusion-induced NE overflow and subsequent cardiac dysfunction. Thus, ET-1 generated from exogenously applied big ET-1 preferentially may act on ET_B receptors rather than ET_A receptors, leading to an increase in NO production and subsequent suppression of NE overflow (Fig. 2).

A study by Khamaisi et al. (60) has shown that ECE-1, a dominant subtype of ECE, colocalizes with the ET_B receptor in the kidney. Accordingly, there is a possibility that ECE-1 and ET_B receptors colocalize somewhere within cardiac tissues. If so, endogenously generated ET-1 should also act preferentially on ET_B receptors. ECE-1 is classified into four isoforms by differences in subcellular distribution in rats (61). Emoto et al. described that the isoform cleaving big ET-1 was different between endogenously synthesized and exogenously supplied ones; endogenous big ET-1 may be cleaved by ECE-1a, which is located intracellularly, whereas exogenously applied big ET-1 may be cleaved by ECE-1b at cell surfaces (62). If ECE1b expressed on the cell surface is

close to ET_B receptors, it is a reasonable explanation as to why ET-1 produced by exogenous big ET-1 preferentially acts on ET_B receptors but not on ET_A receptors. However, further investigations are required to clarify this interesting hypothesis.

According to the results of animal and human studies, the therapeutic potential of ET-receptor antagonists in human heart failure had been tested in randomized clinical trials [REACH-1 (63) and ENABLE (M. Packer, unpublished data): bosentan, RITZ (64–67): tezosentan, HEAT (68) and EARTH (69): darusentan, ENCOR (70): enrasentan]. Most of these studies failed to demonstrate a clear improvement in the clinical status of patients (Table 1) and had to be stopped prematurely because of worsening of the disease or hepatic toxicity (71). Therefore, although these results cast doubt on the usefulness of ET-receptor antagonists for the treatment of myocardial infarction, the pathophysiological role of ET-1, as well as the potential benefit of ET-receptor antagonists, still needs to be investigated in cardiac diseases.

3.2. Balloon angioplasty and neointimal formation

Cardiovascular hypertrophy and remodeling are not simply a response to elevated blood pressure. Various vasoactive substances, such as angiotensin II, are implicated in the development of these structural changes (72).

ET-1 has potent mitogenic and hypertrophic properties, mainly via stimulation of ET_A receptors (73). ET_B receptor-mediated actions also protect against cardiovascular hypertrophy via endothelial NO generation, which inhibits mitogenesis and the proliferation of VSMCs (74).

Balloon angioplasty and stent insertion are now widely used for the treatment of coronary arterial disease. Although these procedures improve regional myocardial blood flow by dilating stenotic coronary vessels, one major drawback of this therapeutic approach is restenosis after the procedure because of the proliferation of VSMCs and neointimal formation triggered by mechanical damage to ECs. Several growth factors or vasoactive peptides are related to the process of neointimal formation. In a clinical study, expressions of ECE, ET-1, and ET receptors were enhanced in neointimal VSMCs after percutaneous coronary intervention in human coronary arteries (75). In addition, increases in ET-1 levels were observed in the coronary circulation after percutaneous transluminal coronary angioplasty (76). Anggrahini et al. recently demonstrated that ET-1 derived from ECs mainly contributes to the process of vascular remodeling in the model of flow cessation (10). Thus, ET-1 is closely related to the pathogenesis of restenosis after angioplasty. Similar results have been reported in animal models with restenosis such as balloon injury (77, 78). Murakoshi et

Table 1. Randomized clinical trials evaluating endothelin receptor antagonists in chronic heart failure

ETR antagonist (selectivity)	Study	Patients	Intervention	Outcome
Bosentan (ET _A /ET _B)	REACH-1	n = 370 NYHA IIIb-IV LVEF < 35%	500 mg, b.i.d., p.o. for 24 wk vs. placebo	No improvements in end points
	ENABLE I ENABLE II	n = 1613 NYHA IIIb-IV LVEF < 35%	62.5 mg, b.i.d., p.o., for 4 wk increased to 125 mg, for an average of 72 wk vs. placebo	No improvements in end points
Tezosentan (ET _A /ET _B)	RITZ-1	n = 675 ADHF	50 mg/h, i.v. for 24 h vs. placebo	No difference in all end points
	RITZ-2	n = 292 ADHF	50 or 100 mg/h, i.v. for 24 h vs. placebo	Both doses produced similar increase in cardiac index and fall in PCWP
	RITZ-4	n = 193 ADHF with ACS	25 mg/h, i.v. for 1 h, then 50 mg/h, i.v. for 23 to 47 h vs. placebo	No difference in all end points
	RITZ-5	n = 84 ACHF	50 or 100 mg/h, i.v. for 24 h vs. placebo	No improvements in end points
Darusentan (ET _A)	HEAT	n = 157 NYHA II LVEF < 35%	10, 100, 300 mg/day, p.o., for 3 wk vs. placebo	Improvement in cardiac index
	EARTH	n = 642 NYHA II-IV LVEF < 35%	10, 25, 50, 100, 300 mg/day, p.o., for 24 wk vs. placebo	No difference in end points
Enrasentan (ET _A /ET _B)	ENCOR	n = 419 NYHA II-III LVEF < 35%	30, 60, 90 mg/day, p.o., for 36 wk vs. enalapril 10 mg/day, p.o., q.i.d. vs. placebo	Better outcome in placebo group

REACH-1: Research on Endothelin Antagonism in Chronic Heart Failure, ENABLE: Endothelin Antagonist Bosentan for Lowering Cardiac Events, RITZ: Randomized Intravenous TeZosentan, HEAT: Heart Failure ET_A Receptor Blockade Trial, EARTH: Endothelin_A Receptor Antagonist Trial in Heart Failure, ENCOR: ENrasentan COoperative Randomized, ETR: endothelin receptor, NYHA: New York Heart Association, ADHF: acute decompensated heart failure, ACS: acute coronary syndrome, ACHF: acute congestive heart failure, LVEF: left ventricular ejection fraction.

al. showed that vascular remodeling caused by the cessation of blood flow was markedly accelerated in the carotid artery of ET_B receptor–knockout mice, and long-term treatment with an ET_B-receptor antagonist worsened vascular remodeling in wild-type mice (6). In contrast, selective ET_A-receptor blockade could attenuate this vascular remodeling in the same animals. There has also been a report showing that ET-1 contributes to the remodeling of mesenteric resistance arteries in diabetes via activation of ET_A receptors, and ET_B receptor-mediated actions provide vasoprotective effects (79). Our previous report has demonstrated that vascular remodeling is markedly attenuated by treatment with a selective ET_A-receptor antagonist, whereas pharmacological blockade of ET_B receptors aggravates neointimal hyperplasia after balloon injury (80). Treatment with an ET_A/ET_B dual receptor antagonist also suppresses neointimal hyperplasia and the efficacy of treatment is comparable with that of a selective ET_A-receptor antagonist (80), thereby suggesting that the antagonism of ET_B receptors does not seem to impair the positive effects of concomitant ET_A-receptor antagonism. Furthermore, we also confirmed similar results in ET_B-deficient rats. Therefore, antagonism of the ET-1 / ET_A receptor pathway appears to be essential for preventing neointimal hyperplasia after balloon injury, irrespective of the presence of ET_B receptor-mediated actions.

3.3. Pulmonary hypertension

The lungs are known to synthesize ET-1 and possess ET_A and ET_B receptors, both of which are involved in physiological and pathophysiological actions of ET-1 in the lung. In particular, endothelial ET_B receptors in lungs are responsible for circulating ET-1 clearance, with close to 50% removal during pulmonary transit in humans (81).

Pulmonary hypertension is characterized by elevated pulmonary arterial pressure, pulmonary arterial remodeling, and right ventricular hypertrophy. ET-1 has been regarded as a major pathological formation and progression factor in pulmonary hypertension because there is a clear correlation between the severity of this disease and increases in the concentration of plasma ET-1 in patients who have primary and secondary pulmonary hypertension (82, 83). Both selective ET_A- and nonselective ET_A/ET_B-receptor antagonists are now widely available for the treatment of pulmonary hypertension at the clinical level (84, 85).

In monocrotaline (MCT)-treated pulmonary hypertensive rats, ET-1 concentrations are higher in their lung perfusate than that of control animals (86). In these rats, cardiac ET-1 mRNA expression and ET-1 peptide levels in heart and plasma are known to be elevated (87, 88). In

addition, elevated ET-1 levels in right ventricular tissue are considered to be induced mainly by pressure overload to the heart rather than the primary causal factor of pulmonary hypertension (88); however, since ET-1 is a potent growth factor of myocardial cells (89), excess ET-1 production in right ventricular tissue seems to promote the progression of right ventricular hypertrophy in pulmonary hypertension. In fact, ET_A-receptor antagonism or ET_A/ET_B-receptor antagonism efficiently attenuated the progression of right ventricular hypertrophy and dysfunction in MCT-induced pulmonary hypertensive rats (87, 90 – 92).

Although the effectiveness of both selective ET_A- and nonselective ET_A/ET_B-receptor antagonists has been demonstrated in human studies, as well as MCT-induced (87, 90 – 92) and hypoxic pulmonary hypertension (92 – 94) in rats, it remains unclear as to which type of selective ET_A-receptor blockade or nonselective ET_A/ET_B-receptor blockade is preferable. In other words, which receptor function of ET_{B1} involved in NO production and ET-1 clearance or ET_{B2} contributing to vascular contractile action should we pay attention to? A study by Nishida et al. (95) showed that blockade of the ET_A receptor inhibited the pathological progression in MCT-induced pulmonary hypertensive rats, but the simultaneous suppression of the ET_B receptor increased the ET_A-receptor antagonist actions. More specifically, combined dosing with an ET_A-receptor antagonist (ABT-627) and ET_B-receptor antagonist (A-192621) exerted similar or more effective actions than that of ABT-627 single dosing against increases in right ventricular systolic pressure and right ventricular hypertrophy by MCT treatment. In addition, increases in the sensitivity of vascular contraction via ET_A and ET_{B2} receptors and reductions in vascular relaxation via the ET_{B1} receptor were observed in the pulmonary vascular bed in this rat model. Furthermore, although pharmacological or genetic inhibition of the ET_B receptor worsened MCT-induced pulmonary hypertension, ABT-627 completely suppressed this deterioration (8, 95). These findings suggest that an ET_A/ET_B-receptor antagonist brings reasonable validity by suppressing overactivation of the ET-1 / ET_A receptor system and vasoconstriction via the ET_{B2} receptor. Moreover, it is less likely that the ET_{B1} receptor actively functions as a protective factor through increases in NO production.

3.4. Salt-sensitive hypertension

Previous studies, including ours, have demonstrated elevations in ET-1 content and ET-1 mRNA expression in vascular tissues of deoxycorticosterone acetate (DOCA)-salt hypertensive rats (96 – 98) and Dahl salt-sensitive rats (99). Long-term treatment with selective

ET_A-receptor antagonists or nonselective ET_A/ET_B-receptor antagonists prevented DOCA-salt-induced hypertension and related tissue injuries, such as vascular hypertrophy, in a qualitatively similar fashion (100 – 102). Although it is unknown which type of antagonist is favorable for the treatment of these hypertensive models, there is general agreement that ET_A receptor-mediated actions play an important role in the development of salt-dependent hypertension and associated tissue injury. On the other hand, we reported that chronic treatment of DOCA-salt rats with A-192621, an orally active and highly potent ET_B-selective receptor antagonist, led to exaggerated deterioration of cardiovascular and renal injuries (102). Other studies using salt-loaded rats also showed that chronic ET_B-receptor blockade indirectly caused activation of the ET-1 / ET_A receptor system followed by increases in blood pressure, thereby suggesting that blockade of the ET_B receptor was harmful in salt-sensitive hypertension (103). In addition, the hypertensive effect induced by an intravenous bolus injection of the selective ET_B-receptor antagonist Ro 46-8443 in DOCA-salt rats was greater than that in uninephrectomized control rats (104). Renal vasoconstrictor effects induced by the selective ET_B-receptor antagonist BQ-788 were also enhanced in DOCA-salt hypertensive rats (105). Our previous study also demonstrated that rats with genetic ET_B deficiency clearly exhibited exaggerated blood pressure sensitivity to DOCA-salt treatment over that in wild-type rats (5). ET_B-deficient rats had enhanced vascular hypertrophy, worsening of renal dysfunction, and tissue damage after DOCA-salt treatment. These changes seen in ET_B-deficient rats were markedly suppressed by the daily administration of ABT-627, a potent ET_A-selective receptor antagonist (5). Furthermore, Elmarakby et al. reported that an ET_A-receptor antagonist attenuated salt-induced hypertension and vascular superoxide production in genetic ET_B deficiency (7). Taken together, ET_B receptor-mediated actions are protective in the pathogenesis of salt-sensitive hypertension. These results also suggest that the antagonism of the ET_A receptor is essential for protection from cardiovascular disease including salt-sensitive hypertension, irrespective of the presence of the ET_B receptor. This view may explain the findings that selective ET_A-receptor antagonists and nonselective ET_A/ET_B-receptor antagonists similarly improve salt-sensitive hypertension and related tissue injuries.

3.5. Sex differences in CVD

Clinical and epidemiological evidence suggests a sexually dimorphic pattern of atherosclerotic CVD in humans. The incidence of CVD is lower in women before menopause than in men and postmenopausal women (106,

107). These sex differences are considered to be caused by the vasoprotective effect of estrogen (108 – 110). In fact, several clinical studies showed that postmenopausal women who receive estrogen replacement therapy (ERT) have a substantially lower risk of incidence of cardiovascular disease (111, 112). The protective effect of estrogen on the cardiovascular system is closely related to the up-regulation of endothelial NO production and down-regulation of adhesion molecule activity, smooth muscle proliferation/migration, and superoxide production (113 – 115). However, recent clinical trials produced different results. The Heart Estrogen-Progestin Replacement Study (HERS) and Women's Health Initiative Clinical Trial and observational study (WHI) did not show any benefit of ERT (116, 117). Thus, the effects of ERT on cardiovascular disease are still controversial. Determinations of the mechanisms of estrogen-exhibited vasoprotective effects and alternative therapies of estrogen in postmenopausal women remain a critical issue.

Substantial evidence indicates that gonadal hormones, especially 17 β -estradiol, modulate the ET system. 17 β -Estradiol inhibits both basal and cytokine-induced ET-1 production at the transcriptional level (118, 119). Vascular functional ECE activity is also affected by 17 β -estradiol (120). In addition, several clinical studies have reported that plasma ET-1 concentrations are lower in women than men (121 – 123). Plasma ET-1 concentrations in women fluctuate during the menstrual cycle (124) and pregnancy (125). Healthy older women exhibit higher plasma ET-1 levels than those of healthy young or middle-aged women. Hormone replacement therapy (17 β -estradiol plus methoxyprogesterone) in healthy postmenopausal women results in decrease in plasma ET-1 levels (122). Furthermore, an investigation of cross-gender hormone treatment indicated that plasma ET-1 levels in male-to-female transsexual patients were decreased (126). Taken together, it is reasonable to assume that 17 β -estradiol is mainly involved in sex differences in the ET system.

Sex differences in ET-receptor density, as well as in the ratio of ET-receptor subtypes, have been also investigated. Ergul et al. reported that men's saphenous veins have a larger number of ET receptors and an increased ratio of ET_A to ET_B receptors compared to women's saphenous veins and that these differences were reflected by the sex differences in ET-1-induced vascular contractile responses (127). On the other hand, although several animal studies also indicated that ET receptors are involved in the sex differences in the incidence of CVD, the effect of estrogen on these receptors is quite contradictory. For example, Nuedling et al. demonstrated up-regulation of the ET_B receptor in the heart of ovariectomized female spontaneously hypertensive rats, which

could be reversed by exogenous estrogen replacement (128). They also confirmed downregulation of the ET_B receptor by 17 β -estradiol in cultured cardiomyocytes. Others reported similar results showing that vascular mRNA expression of ET_B, but not ET_A receptors in DOCA-salt-induced hypertensive rats was higher in males than that observed in females (129, 130). In contrast, Pedersen et al. showed that 17 β -estradiol treatment reduced levels of the ET_A, but not the ET_B receptor in the rabbit thoracic aorta and epicardial arteries (131). However, their previous study indicated that 17 β -estradiol induced up-regulation of ET_B-receptor gene expression in coronary arteries from ovariectomized hyperlipidemic rabbits (132). Thus, estrogen may cause differential effects on vascular ET receptors in different species and/or vascular beds.

Our previous study demonstrated that the frequency of neointimal formation after balloon injury was much lower in females than in males and that neointimal formation after vascular injury in female rats was significantly aggravated by ovariectomy, and this aggravation was markedly improved by 17 β -estradiol treatment (133). These results clearly indicate that estrogen inhibits neointimal formation after vascular injury in female rats. Furthermore, this sex difference in neointimal formation was abolished by genetic ET_B-receptor deficiency or pharmacological ET_B-receptor blockade. Both ovariectomy and ovariectomy plus 17 β -estradiol treatment failed to affect the enhanced neointimal formation observed in intact female ET_B-deficient rats. In other words, as the vasoprotective effects of estrogen after vascular injury were abolished by genetic deficiencies in the ET_B receptor, estrogen likely reduces neointimal formation after vascular injury via a mechanism that depends on ET_B receptor-mediated actions. These findings suggest that ET_B receptor-mediated actions seem to occur downstream of the vasoprotective effects of estrogen, although the relationship between ET_B receptor- and estrogen receptor-signaling systems remains unclear. On the other hand, neointimal hyperplasia observed in female ET_B-deficient rats is almost completely suppressed by ET_A or ET_A/ET_B-receptor antagonists. Thus, augmentation of ET_A receptor-mediated actions under ET_B-receptor dysfunction seems to be responsible for the abolition of sex differences in vascular remodeling.

4. Conclusion

Since the discovery of ET-1, many researchers have elucidated the physiological and pathophysiological role of ET-1 and ET receptors in the cardiovascular system over the past 20 years. Among many non-peptide and orally available ET-receptor antagonists developed so

far, the nonselective ET_A/ET_B-receptor antagonist bosentan and selective ET_A-receptor antagonist ambrisentan are now clinically utilized as agents for pulmonary artery hypertension. There is a possibility that ambrisentan could be widely used in the treatment of pulmonary hypertension because of less interactions with other drugs or side effects such as liver dysfunction. In addition, future clinical applications may provide new findings about which antagonist is more effective, a nonselective ET_A/ET_B-receptor or selective ET_A-receptor antagonist. On the other hand, although the selective ET_A-receptor antagonist sitaxsentan, which was released in Europe and the United States, was recently forced to be withdrawn because of a high risk of liver failure, it is hoped in the future that other ET-receptor antagonists, including macitentan and zibotentan, currently being developed can be utilized in clinical treatment targeted at the cardiovascular ET-1 system.

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