Endothelin, sex and hypertension

Rita C. TOSTES*, Zuleica B. FORTES*, Glauca E. CALLERA‡, Augusto C. MONTEZANO‡, Rhian M. TOUYZ‡, R. Clinton WEBB† and Maria Helena C. CARVALHO*

*Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, 05508-900 Sao Paulo, Brazil, †Department of Physiology, Medical College of Georgia, Augusta, GA 30912-3000, U.S.A., and ‡Kidney Research Centre, Ottawa Health Research Institute, University of Ottawa, Ottawa, Canada K1H 8M5

ABSTRACT

The ETs (endothelins) comprise a family of three 21-amino-acid peptides (ET-1, ET-2 and ET-3) and 31-amino-acid ETs (ET-11−31, ET-21−31 and ET-31−31). ET-1 is synthesized from a biologically inactive precursor, big ET-1, by ECEs (ET-converting enzymes). The actions of ET-1 are mediated through activation of the G-protein-coupled ETA and ETB receptors, which are found in a variety of cells in the cardiovascular and renal systems. ET-1 has potent vasoconstrictor, mitogenic, pro-inflammatory and antinatriuretic properties, which have been implicated in the pathophysiology of a number of cardiovascular diseases. Overexpression of ET-1 has been consistently described in salt-sensitive models of hypertension and in models of renal failure, and has been associated with disease progression. Sex differences are observed in many aspects of mammalian cardiovascular function and pathology. Hypertension, as well as other cardiovascular diseases, is more common in men than in women of similar age. In experimental models of hypertension, males develop an earlier and more severe form of hypertension than do females. Although the reasons for these differences are not well established, the effects of gonadal hormones on arterial, neural and renal mechanisms that control blood pressure are considered contributing factors. Sex differences in the ET-1 pathway, with males displaying higher ET-1 levels, greater ET-1-mediated vasoconstrictor and enhanced pressor responses in comparison with females, are addressed in the present review. Sex-associated differences in the number and function of ETB receptors appear to be particularly important in the specific characteristics of hypertension between females and males. Although the gonadal hormones modulate some of the differences in the ET pathway in the cardiovascular system, a better understanding of the exact mechanisms involved in sex-related differences in this peptidergic system is needed. With further insights into these differences, we may learn that men and women could require different antihypertensive regimens.

INTRODUCTION

According to the Department of Reproductive Health and Research, World Health Organization (WHO Draft working definition, October 2002; http://www.who.int/reproductive-health/gender/glossary.html), “sex refers to the biological and physiological characteristics that define humans (and animals) as male and female, whereas gender

Key words: arterial hypertension, endothelin, endothelin receptor, end-organ damage, 17β-oestradiol, sex, testosterone.

Abbreviations: ACE, angiotensin-converting enzyme; AngII, angiotensin II; AT1 receptor, AngII type 1 receptor; BP, blood pressure; CABG, coronary artery bypass graft; COX, cyclo-oxygenase; DAG, diacylglycerol; DOCA, deoxycorticosterone acetate; ERK, extracellular-signal-regulated kinase; ER, oestrogen receptor; ET, endothelin; ECE, ET-converting enzyme; HUVEC, human umbilical vein endothelial cell; IL, interleukin; IP3, inositol trisphosphate; JAK2, Janus kinase 2; MAPK, mitogen-activated protein kinase; NC-IUPHAR, International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification; NF-κB, nuclear factor κB; NOS, nitric oxide synthase; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; Pyk2, proline-rich tyrosine kinase 2; ROS, reactive oxygen species; SHR, spontaneously hypertensive rat; SHRSF, stroke prone SHR; sl, spotting-lethal; STAT, signal transducer and activator of transcription.

Correspondence: Dr Rita C. Tostes (email rtostes@mcg.edu).
refers to the economic, social and cultural attributes and opportunities associated with being male or female in a particular point in time." Therefore, in the present review, which focuses on differences in the ET (endothelin) system between male and female subjects, the word sex will be used throughout the text. Since the gonadal hormones co-ordinate several integrated responses in the cardiovascular system and because many of these processes represent characteristic features of ET-1 actions, the effects of gonadal hormones in the ET system will also be discussed.

ENDOTHELIN

In this section, a brief overview of the discovery of ET, mechanisms of synthesis, ET receptors and its main actions on the renal and cardiovascular systems will be addressed. Readers will be referred to recent reviews on these topics.

The ET family

In 1985, Hickey and co-workers [1] described the existence of a trypsin-sensitive endothelium-derived constrictor factor in cultured bovine endothelial cells. Subsequently, 3 years later, Yanagisawa and co-workers [2] isolated, purified, cloned and sequenced the 21-amino-acid vasoconstrictor peptide, then named ET-1. Subsequently, two other ET-1 isoforms, ET-2 and ET-3 [3], along with two receptor subtypes, ET_A [4] and ET_B [5], and the enzymes responsible for ET-1 synthesis, ECE-1 (ET-converting enzyme-1) [6,7] and ECE-2 [8], were identified. At the present time, the ET family comprises four endogenous isoforms of 21-amino-acid peptides, ET-1, ET-2 and ET-3 and ET-4 [or VIC (vasoactive intestinal peptide)].

ET synthesis

The two intramolecular disulfide bonds, between cysteine residues cross-linked at positions 1 and 15 and 3 and 11, and the dual secretory pathways are unique characteristics of ET-1 among the mammalian bioactive peptides [1,14,15]. ET-1 is released continuously from vascular endothelial cells by a constitutive pathway, producing intense constriction of the underlying smooth muscle and contributing to the maintenance of endogenous vascular tone. ET-1 is also released from endothelial cell-specific storage granules (Weibel-Palade bodies) in response to external physiological stimuli, producing further vasoconstriction [14]. In addition to endothelial cells, ET-1 is produced by other cell types in the renal and cardiovascular systems, such as smooth muscle cells, cardiomyocytes, leukocytes, macrophages, and renal tubular and mesangial cells and is secreted abuminally [15–18]. Because ET-1 concentrations are lower in plasma in comparison with endothelial and other cells, it is accepted that ET-1 functions as a locally released, rather than circulating, hormone.

Bioactive ETs are the product of post-translational processing of the parent pre-pro-ET peptide. Transcription of the pre-pro-ET gene and translation of pre-pro-ET mRNA results in the formation of a 203-amino-acid peptide, which is later cleaved by a furin convertase to the 38-amino-acid peptide big ET1 [9]. Big ET is processed further into ET1 [21] by different isoforms of ECEs, a group of proteases that belong to the metalloprotease family and share both structural and functional similarity with neutral endopeptidases and Kell blood group proteins [19,20] (Figure 1). In mammals, there are four isoforms of ECE-1 (ECE-1a, ECE-1b, ECE-1c and ECE-1d), derived from a single gene by the action of alternative promoters. They are located mainly on the plasma membrane or within intracellular compartments and differ only in the amino acid sequence of the extreme N-terminus. Levels of mRNA encoding ECE-1 are relatively low in tissues where endothelial cells represent only a small proportion of the cell type. Accordingly, ECE-1 levels are higher in the lungs and kidneys compared with levels in the heart and brain. ECE-2 is a membrane-bound metalloprotease with 59% homology with bovine ECE-1, but with distinct biochemical properties (higher activity in acidic pH and more sensitive to phosphoramidon inhibition). Four isoforms of ECE-2 (ECE-2a-1, ECE-2a-2, ECE-2b-1 and ECE-2b-2) have been identified. The ECE-2 isoforms also differ in their N-terminus and are associated with secretory vesicles [15,20]. Bioactive 31-amino-acid-length ETs, ET1 [31], are synthesized by chymases (chymotrypsin-like serine proteases present in large quantities in mast and smooth muscle cells that selectively cleave big ET-1, ET-2 and ET-3 at their Tyr31-Gly32 bonds [9,19] (Figure 1). Moreover, non-ECE metalloproteases, such as MMP-2 (matrix metalloproteinase-2), are involved in big ET-1 metabolism, resulting in the formation of ET1 [31], a peptide that also exhibits potent vasoconstrictor effects.

ET-1 is the principal isoform in the human cardiovascular system and is the most potent and unusually long-lasting constrictor of human vessels known to date [2].
ET-1 synthesis is controlled via regulation of gene transcription and/or ECE activity and its release occurs mostly abluminally. ET-1 acts on ETA and ETB smooth muscle receptors to induce vasoconstrictor, pro-oxidant, pro-inflammatory and mitogenic responses. ET-1 also acts in an autocrine manner, at endothelial cell ETB receptors, to stimulate the release of vasorelaxant agents such as NO and PGI2 (prostaglandin I2). ETB receptors present on kidney and lung endothelial cells also remove ET-1 from the circulation, functioning as clearance receptors. AA, arachidonic acid; eNOS, endothelial NOS.

ET-2, which differs by only two amino acids from ET-1, is also a potent vasoconstrictor and has been detected in cells of the cardiovascular system. ET-3, which at physiological concentrations has little or no affinity for the ETA receptor subtype, is detectable in the plasma, heart, brain and adrenal gland, but not in endothelial cells [15–18].

**ET receptors**

On the basis of the cellular localization within the vasculature, it was initially suggested that vascular smooth muscle cell ETA receptors mediate vasoconstriction and endothelial ETB receptors elicit vasodilation through the release of endothelium-derived relaxing factors. However, it was later shown that both ETA and ETB receptors mediate constriction of various vascular beds, including arteries and veins. Normally, ETA receptors predominate in vascular smooth muscle cells, whereas a low density of ETB receptors is observed [15–18] (Figure 1).

Binding sites for both ETA and ETB receptors are widely and heterogeneously distributed throughout the human heart, including myocardial muscle and the ativoventricular conducting system. The ETA receptor is the predominant subtype in adult cardiac myocytes, and a mixed population of ETA and ETB receptors has been described in cardiac fibroblasts and in endocardial endothelial cells [21–23]. Renal ETB receptors represent the major population of ET receptors (70% of the receptors in both cortex and medulla), localizing both to endothelial cells and non-vascular tissues (tubules and collecting ducts) [31–33]. ETA receptors are localized to vascular smooth muscle of arteries and veins as well as intra-renal resistance vessels and, probably due to the high density of ET receptors, the renal vasculature is highly sensitive to the pre- and post-glomerular vasoconstrictor actions of ET-1 [18,24,25]. In addition to the tissues and organs that are part of the renal and cardiovascular systems, ET receptors are widely expressed in almost all systems.

The ETB receptors present on endothelial cells also remove ET-1 from the circulation, functioning as clearance receptors [26–28] (Figure 1). Blockade of the ETB receptor results in a rise in circulating immunoreactive ETs (ET-1 and ET-3), whereas mice with genetic ablation of the ETB receptor in endothelial cells exhibit elevated plasma concentrations of ET-1 [15,26,27]. Furthermore, infusion of radiolabelled ET-1 leads to a rapid accumulation of ET-1 in the lung, kidney and liver, but not in the heart. Infusion of an ETB-selective antagonist, BQ788, before administration of radiolabelled ET-1 blocks the binding of the radioligand peptide to ETB receptors in the kidney and lung, and, in the presence of ETB blockade, ET-1 is able to rapidly bind to cardiac ETA receptors [28].
ET receptors, initially classified as ET<sub>A</sub> and ET<sub>B</sub> by NC-IUPHAR (International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification) [29], were tentatively subclassified in ET<sub>B1</sub> and ET<sub>B2</sub> subtypes. However, on the basis of the many studies addressing the expression of ET receptors and the effects of ET receptor antagonists, the latest NC-IUPHAR consolidated the classification of mammalian ET receptors as ET<sub>A</sub> and ET<sub>B</sub> receptors [30]. Pharmacologically heterogeneous responses to ET<sub>A</sub> or ET<sub>B</sub> agonists seem to be related to the existence of alternatively spliced variants of the ET<sub>A</sub> and ET<sub>B</sub> receptors. A gene encoding a third receptor, named ET<sub>C</sub> and reported to be specific for ET-3 binding, was cloned from dermal melanophores from the amphibian *Xenopus laevis*, but a mammalian homologue has not yet been identified [30].

Both ET<sub>A</sub> and ET<sub>B</sub> receptors belong to the seven-transmembrane domain or G-protein-coupled rhodopsin-type receptor superfamily. Stimulation of ET receptors in smooth muscle cells results in PLC (phospholipase C) activation, which leads to the generation of the second messengers IP<sub>3</sub> (inositol trisphosphate) and DAG (diacylglycerol), which can, in turn, stimulate intracellular Ca<sup>2+</sup> release and activate several isoforms of PKC (protein kinase C) respectively [31]. Other signalling pathways are also activated and involve PLD (phospholipase D) and DAG generation, PLA<sub>2</sub> (phospholipase A<sub>2</sub>) stimulation and arachidonic acid release, activation of the Na<sup>+</sup>/H<sup>+</sup> exchanger, activation of the MAPK (mitogen-activated protein kinase) family (ERK1/2, c-Jun N terminal kinases and p38 MAPKs), Pyk2 (proline-rich tyrosine kinase 2), PI3K (phosphoinositide 3-kinase) and JAK2/STAT (Janus kinase 2/signal transducer and activator of transcription). Activation of all of these signalling pathways is involved in the short-term regulation of muscle tone as well as in the long-term control of cell growth, adhesion, migration and intercellular matrix deposition in cells from the vasculature, kidney and heart [31,32]. Activation of endothelial cell ET<sub>A</sub> receptors stimulates the release of NO and prostacyclin, negatively modulating the constrictor effects of ET-1 on smooth muscle cells [27] (Figure 1). Thus the overall effect of ET-1 on vascular tone derives from the balance between the direct vasoconstrictor effect via ET<sub>A</sub> and ET<sub>B</sub> receptors on smooth muscle cells and NO- or prostacyclin-induced vasodilatation mediated by endothelial ET<sub>A</sub> receptors.

An interesting aspect of ET-1 is its ‘irreversible’ binding characteristic. The tenacious binding of ETs (mainly ET-1 and ET-3) to their receptors may explain the long-lasting vasoconstrictor effects of these peptides. Some hypotheses, such as a peculiar interaction between ET receptors and G-proteins, disulfide interchange in the ET receptor–ligand complex and a continuous ET receptor externalization, have been proposed to explain the stability of the ligand–receptor complex [2,33].

### ET actions in the cardiovascular and renal systems

ET-1 not only induces vasoconstriction, but it is also a potent growth-promoting and pro-inflammatory agent. ET-1 stimulates the production of growth factors as well as the deposition of extracellular matrix components, such as collagen and fibronecin; it induces generation of ROS (reactive oxygen species), activates nuclear factors [such as NF-κB (nuclear factor κB)] and stimulates the expression of adhesion molecules by endothelial cells; and it potentiates monocyte migration, activates transcriptional factors responsible for the co-ordinated increase in the expression of many cytokines [for example, IL-6 (interleukin-6), MCP-1 (monocyte chemoattractant protein-1) and IL-8] and enzymes (iNOS [inducible NOS (nitric oxide synthase)], COX (cyclo-oxygenase) and NADPH oxidase), which can, in turn, lead to the production of inflammatory and tissue damage mediators [31,32]. In addition, ET-1 potentiates the action of other vasoconstrictors, such as AngII (angiotensin II), phenylephrine and serotonin [31,32].

In the heart, depending on the animal species and experimental conditions, ET-1 induces either an increase or a decrease in myocardial contractility. In most species, although the activation of ET<sub>A</sub> receptors is responsible for an increase in contractility, the activation of ET<sub>B</sub> receptors causes the opposite effect [23]. However, in an open-chest rat model, ET-1 caused an increase in contractility when given after ET<sub>A</sub> receptor blockade, an effect associated with the stimulation of cardiac ET<sub>B</sub> receptors and not only to changes in vascular haemodynamics, suggesting that ET<sub>A</sub> blockade unmasks the ET<sub>B</sub>-receptor-mediated positive inotropic effect of ET-1 [23]. Whether endogenous ET-1 (produced by both endocardial endothelial cells, which line the cavities of the heart, and the microvascular endothelial cells, which line the blood vessels of the heart) has a physiological role in regulating contractile function is likely to depend on the levels of peptide achieved in the vicinity of the myocytes and the modulatory influences of other myocardial or vasoactive factors released from the nerves and the vascular or endocardial endothelium [17]. The increase in contractility appears to be associated with activation of PLC, PKC, Na<sup>+</sup>/H<sup>+</sup> exchanger and increased cytoplasmic Ca<sup>2+</sup> levels, whereas the ET-1-induced negative inotropic effect is associated with activation of PKG (protein kinase G), via a pertussis-toxin-sensitive G<sub>i</sub>-protein, inhibition of adenylyl cyclase, reduced cAMP levels and decreased cytoplasmic Ca<sup>2+</sup> [17,23].

In the kidneys, ET-1 exerts a wide variety of biological effects, including constriction of cortical and medullary vessels, mesangial cell contraction, stimulation of extracellular matrix production and inhibition of sodium...
and water reabsorption along the collecting duct, effects that are primarily mediated in an autocrine/paracrine manner. Renal ET \( \beta \) receptors, which represent the major population of ET receptors in both the cortex and medulla, mediate natriuresis by different mechanisms: renal tubular ET \( \beta \) receptors inhibit reabsorption of sodium in the thick ascending limb and collecting duct, whereas medullary ET \( \beta \) receptors enhance the excretion of salt and water under conditions of high salt intake through vasodilation within the renal medulla [24,25].

**Pathways to inhibit the ET system**

ET antagonists are classified as ETA-selective, ETB-selective or mixed (non-selective) ETA/ETB antagonists and a number of both peptide and non-peptide antagonists are available or in clinical development [15,16]. Considering the opposing actions mediated by ETA and ETB receptors (mainly in the vasculature and kidneys), differential effects are expected with these drugs, and the effects of ET \( \beta \) antagonists might be harmful. Possibly reflecting the lack of clinical need for ET \( \beta \) antagonists, very few peptide or non-peptide ET \( \beta \) antagonists have been developed.

ECE inhibition diminishes ET-1 production and, consequently, the effects of ET-1. However, the effectiveness of these drugs are limited by two factors: (i) the identification of ECE-independent pathways contributing to ET-1 formation, such as chymases and non-ECE metalloproteases, and (ii) the overlapping of the different isoforms of ECE for ET-1 production [34]. Another important aspect to be considered is that the indirect inhibition of ET-1 production by renin-angiotensin system inhibitors [35], given the fact that Ang II-related vascular effects are partly mediated by ET-1. In this sense, the antihypertensive efficacy of ETA/ETB blockade is augmented by AT\(_1\) receptor (Ang II type 1 receptor) blockade in hypertensive rats [36], whereas ETA receptors (mainly in the vasculature and kidneys), differential effects are expected with these drugs, and the effects of ET \( \beta \) antagonists might be harmful. Possibly reflecting the lack of clinical need for ET \( \beta \) antagonists, very few peptide or non-peptide ET \( \beta \) antagonists have been developed.

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**HYPERTENSION**

**Pathophysiological role of ET-1 in hypertension**

In hypertension and other cardiovascular diseases, activation of the ET system is inferred from measurements of ET-1 plasma levels, tissue ET-1 gene expression, ET-1 peptide abundance and response to ET-1 antagonists.

Increased ET-1 plasma levels are observed only in severe and accelerated forms of human essential hypertension and in patients exhibiting impaired renal clearance [38–40]. However, ET-1 plasma values may not reflect local increased production of the peptide. Additionally, mean vasoconstriction in response to exogenous ET-1 is significantly increased in hypertensive patients compared with normotensive controls, and it is positively correlated with BP values in hypertensive patients, but not in control subjects [41]. Furthermore, infusion of low concentrations of ET-1 causes a slight vasodilation, shifting to vasoconstriction at higher infusion rates in control subjects [42], whereas in essential hypertensive patients ET-1 causes vasoconstriction at the lowest infusion rates. An increased vasoconstrictor response to a single dose of ET-1 has also been observed in essential hypertensive patients compared with normotensive controls [44]. Consistent with the possible down-regulation of the ETA receptor in hypertension, reduced forearm vasoconstriction to ET-1, but not to sarafotoxin S6c, was also reported in patients with essential hypertension [45].

Extensive results demonstrate that ET-1 is important in salt-sensitive forms of experimental hypertension, such as DOCA (deoxycorticosterone acetate)-salt hypertensive rats, DOCA-salt-treated SHR rats, Dahl salt-sensitive rats, Ang II-infused rats, renovascular two-kidney one-clip Goldblatt rats, SHRSP without or with salt loading, aldosterone-infused rats and dTGRs (double transgenic rats) harbouring the human renin and angiotensinogen genes [16,32,46]. In these experimental models, ET receptor antagonists have moderate BP-lowering efficacy and are not effective antihypertensive agents. However, treatment of these animals with both selective ETA or dual ETA/ETB receptor antagonists results in regression of vascular damage and endothelial dysfunction, delayed progression of renal injury, cerebral oedema reduction, cardiac and vascular hypertrophy regression and survival improvement [32,46].

Since the beneficial actions of ETA antagonists occur in the absence of marked BP-lowering, ET-1 probably has direct cardiovascular and renal actions dissociated from its systemic haemodynamic effects. The pro-inflammatory and pro-growth characteristics of ET-1 are major candidates for the cardiovascular and renal effects of ET-1 [32,46–51]. Additionally, ET-1, via activation of both ETA or ETB receptors (depending on the cell type), directly stimulates superoxide production. ET-1-induced increases in superoxide production are mediated by NADPH-oxidase-dependent and -independent (uncoupling of NOS and mitochondrial-derived
ROS) mechanisms [52–56]. Consistent with a direct non-haemodynamic effect of ET-1-derived superoxide in end-organ damage (inflammatory and fibrotic processes), mice with targeted overexpression of human pre-pro-ET-1 in vascular endothelial cells exhibit structural remodelling and endothelial dysfunction of resistance vessels, in part through the activation of vascular NADPH oxidase, but not changes in BP [57].

Reinforcing these deleterious actions of ET-1 on hypertension-associated end-organ damage, blockade of ET-1 receptors in these models of hypertension alleviated both renal and cardiovascular damage by decreasing inflammation-related processes, such as the activation of NF-κB and AP-1, macrophage infiltration, ICAM and VCAM (intercellular and vascular cell adhesion molecules respectively) expression and leucocyte–endothelium interactions as well as oxidative stress or superoxide generation [32,46–51,55,56].

**Therapeutic potential of ET receptor antagonists in hypertension**

Available results with ET receptor antagonists show that, in healthy subjects, ETA antagonists alone or in combination with ETB antagonists do not significantly modify basal vascular tone, whereas, in essential hypertensive patients, ETA receptor blockade causes a slight vasodilation that is potentiated by ETB receptor blockade. In line with a pathophysiological role of ET-1 in essential hypertension, one of the earliest clinical studies showed that long-term administration of the mixed ETA/ETB antagonist bosentan significantly lowered BP in patients with essential hypertension [16,40]. Small clinical studies with other ET-1 antagonists, such as TAK-044 (non-selective antagonist) and sitaxsentan (ETA selective antagonist), also support a role for ET-1 antagonists in hypertension, and clinical trials with the selective ETA antagonist darusentan are showing promising results in patients with resistant hypertension [16,32,46].

Owing to the predominant role of ET-1 in salt-sensitive forms of hypertension, ET-1 antagonists may be particularly beneficial in this condition. Accordingly, the vasodilator effect upon ETA receptor blockade is significantly higher in African-American hypertensive patients, which usually present with a salt-sensitive low-renin form of hypertension, than in Causcasian hypertensive patients [38]. Significantly higher vasodilator responses to ETA receptor blockade are also observed in obese and overweight hypertensive patients in comparison with lean hypertensive patients [59]. In hypertensive patients, but not in control subjects, a significant correlation between BMI (body mass index) and the vasodilator response to ETA blockade was observed [59], which is in close association with results from experimental studies showing that ET-1 activity is enhanced in insulin-resistant states [32]. Although evidence to support a role for ET-1 antagonists in arterial hypertension exists, the therapeutic potential of these agents in the management of hypertension must be validated by large clinical trials.

**SEX**

Sex differences are observed in many aspects of mammalian cardiovascular function and pathology. Although the reasons for these differences are not well established, the effects of gonadal hormones on arterial, neural and renal mechanisms that control blood flow are considered contributing factors. In this section, the greater incidence of hypertension, as well as hypertension-related organ damage, in males than females will be discussed. Evidence for sex differences in the components of the ET system as well as the modulatory actions of gonadal hormones will be discussed.

**Sex differences in hypertension**

Hypertension, as well as other cardiovascular diseases, is more common in men 30–45 years of age than in women of a similar age [60–63]. In experimental models of hypertension, males develop an earlier and more severe form of hypertension than do females [64,65]. Similarly, in chronic renal conditions, such as membranous nephropathy, polycystic kidney disease or nephritic syndrome, men progress more rapidly in their diseases than women [66,67]. Males also develop greater age-related renal injury, despite similar levels of BP, and proceed to end-stage renal failure faster than do females [68].

Hypertension is also more prevalent in postmenopausal than premenopausal women, and premenopausal females are relatively protected against the progression of renal diseases when compared with postmenopausal women [62,68]. Although endogenous androgens appear to play a role in mediating hypertension and renal injury not only in males, but also in females with relatively high androgen levels, for example at the time of menopause [68–70], ovarian hormones, mainly 17β-oestradiol, are believed to display protective effects on the cardiovascular and renal systems [71–74]. Indeed, significant effects of sex hormones on the cardiovascular, neuronal and renal control mechanisms of BP have been proposed [68–74].

**Sex differences in the ET system and the modulatory effects of gonadal hormones**

Sex differences in the ET-1 pathway have been shown at different levels: ET-1 plasma or tissue concentration, ET-1 production, ET-1 receptors, ET-1-induced effects and responses to ET-1 receptor antagonists (Figure 2). In many cases, gonadal hormones appear to modulate the sex-related differences in the ET system, as will be discussed.

**Plasma and tissue levels of ET**

Substantial evidence indicates that females have lower ET-1 levels due to a modulatory action of female
Figure 2  Sex-related differences in the ET system
Gene regulation at the transcriptional level and ECE activity seem to contribute to sex-related differences in ET-1 production, with females having lower plasma and tissue ET-1 levels than males. Sex also influences ET-1 responses, with males exhibiting greater vasoconstrictor, pressor, mitogenic and inflammatory responses in comparison with females, and expression or function of ET-1 receptor subtypes, with males exhibiting increased number/function of smooth muscle ETA and ETB receptors and females having greater activation of endothelial cell (and clearance) ETB receptors.

gonadal hormones. Plasma ET-1 levels are higher in men than in age-matched women, both in physiological and pathological conditions [75,76]. Plasma ET-1 levels fluctuate during the menstrual cycle, with lowest ET-1 levels during the follicular and luteal phase compared with the menstrual (low-oestrogenic) phase [77]. ET-1 also decreases during pregnancy (when oestrogen levels are high) [75,78,79] and during oestrogen administration in transsexual male patients (administration of ethynylestradiol and cyproterone acetate to men) [75]. In addition, plasma ET-1 concentration is markedly higher in healthy older women than in healthy young or middle-aged women [80]. Healthy postmenopausal women on continuous hormone replacement therapy (17β-oestradiol combined with norethisterone acetate or methoxyprogesterone) exhibit decreased ET-1 levels and increased plasma nitrites/nitrates [81]. Women receiving the selective oestrogen receptor modulator raloxifene also have similar changes in plasma ET-1 and nitrites/nitrates levels, as well as in the ratio of NO to ET-1 [82].

The possibility that testosterone modulates ET-1 levels is supported by the observation that female-to-male transsexuals (women treated with testosterone) have increased ET-1 levels [75,83]. In addition, obese and non-obese women with polycystic ovary syndrome (who present with hyperandrogenaemia, hyperinsulinaemia and insulin resistance) have higher levels of ET-1 compared with obese or non-obese healthy, normal cycling, age-matched women [84,85]. A positive correlation of ET-1 with free testosterone levels was also shown in these studies [84,85]. However, the suggestion is weakened by results showing that ET-1 levels in patients with various forms of hypogonadism are significantly higher in comparison with age-matched healthy males and that testosterone therapy decreases ET-1 levels in these individuals [86].

Sex differences in plasma and tissue levels of ET-1, with females displaying lower ET-1 levels, have also been described in experimental animal models in physiological and pathological conditions. In DOCA-salt hypertensive rats, a model where ET-1 plays a major role in the pathogenesis of hypertension [16,32], male hypertensive animals have increased vascular pre-pro-ET-1 mRNA in comparison with hypertensive females [87,88]. Removal of the ovaries and, consequently, a significant reduction in oestradiol and progesterone levels, increases vascular pre-pro-ET-1 mRNA in comparison with hypertensive females [87,88]. Removal of the ovaries and, consequently, a significant reduction in oestradiol and progesterone levels, increases vascular pre-pro-ET-1 mRNA in comparison with hypertensive females [87,88]. Removal of the ovaries and, consequently, a significant reduction in oestradiol and progesterone levels, increases vascular pre-pro-ET-1 mRNA in comparison with hypertensive females [87,88].

Expression of pre-pro-ET and ECE activity/expression
Mechanisms associated with sex differences in ET-1 levels appear to involve gene regulation at the transcriptional level, mainly by the ovarian hormones. 17β-Oestradiol...
inhibits both basal and induced ET-1 mRNA expression in various cell types, including endothelial cells, smooth muscle cells, cardiac myocytes, fibroblasts and mesangial cells, and species [91–98]. Accordingly, incubation of cultured HUVECs (human umbilical vein endothelial cells) [91] or cultured bovine carotid arterial endothelial cells [92] with 17β-oestradiol inhibits both ET-1 mRNA expression and ET-1 release. Aortic endothelial cells from ovariectomized Yorkshire pigs exhibit increased pre-pro-ET-1 mRNA expression in comparison with cells from gonadally intact male and female pigs [93]. 17β-Oestradiol also inhibits strain-induced ET-1 gene expression, associated with increases in intracellular ROS generation and ERK phosphorylation, in cultured endothelial cells [94]. 2-Hydroxyoestradiol or 2-methoxyoestradiol, metabolites of oestradiol with little or no affinity for ERs (oestrogen receptors), inhibit ET-1 synthesis in porcine coronary artery endothelial cells, possibly via inhibition of MAPK activity [95]. In addition, 17β-oestradiol inhibits AngII-induced ET-1 gene expression in rat aortic smooth muscle cells and cardiac fibroblasts [96,97]. Accordingly, ET-1 expression is higher in aortic smooth muscle cells from ovariectomized rats and 17β-oestradiol replacement decreases ET-1 levels [98].

Oestradiol seems to modulate both ET-1 gene expression and production directly and indirectly. Because it is well known that oestradiol increases NO levels and NOS activity [73] and inhibition of NOS partly attenuates the effect of 17β-oestradiol on ET-1 gene expression in HUVECs [92], NO is a major candidate for the indirect effects of oestradiol. In addition, both ER-mediated [92,94,96,97] and ER-independent [95] mechanisms appear to be involved in the inhibitory effects of oestradiol on ET-1 expression.

Vascular functional ECE activity is increased in ovariectomized rats, and treatment with oestrone reduces ECE activity [99], suggesting that oestradiol-induced inhibition of ET-1 production is also mediated by changes in ECE activity. Furthermore, oestradiol, as well as the phytoestrogens genistein and daidzein, inhibit ECE-1 mRNA expression in rat mesenteric artery by an ER-mediated mechanism [100]. In human internal mammary arteries and saphenous veins, obtained from patients undergoing CABG (coronary artery bypass graft) surgery, oestradiol exerts opposite effects on ECE activity (calculated as the contraction to big ET-1/contraction to ET-1 ratio). Although oestradiol suppresses ECE activity in saphenous veins, it markedly enhances ECE activity in internal mammary arteries [101].

To date, there is no evidence for a regulatory role of testosterone on ECE activity in the cardiovascular system.

**Effects of ET on the cardiovascular and renal systems**

Sex-related differences in ET-1 responses have also been reported. ET-1-induced contractions are 2-fold higher in saphenous vein samples obtained from male patients undergoing CABG surgery than in females [102]. Administration of the ETα receptor antagonist BQ-788 into the forearm skin of human subjects, by intradermal microdialysis, revealed that, in men, ETα receptors mediate tonic vasoconstriction, whereas, in women, ETβ receptors mediate tonic vasodilation [103].

We have reported that both conductance and resistance arteries from male, but not female, DOCA-salt rats have increased sensitivity to ET-1 and to the selective ETβ receptor agonist IRL-1620 [104,105]. In vivo IRL-1620 induces vasodilation in mesenteric microvessels from control rats, marked vasoconstriction in male DOCA-salt rats and a very mild constriction in vessels from female DOCA-salt rats [105]. Changes in ETβ-mediated vascular responses are associated with increased ET-1 and ETβ receptor gene expression in male, but not in female, animals [88]. Furthermore, ovariectomy increases the vasoconstrictor responses to the ETβ agonist, and oestrogen-replacement therapy restores IRL-1620-induced responses [87], reinforcing the suggestion that the ovarian hormones modulate ET-1/ETβ receptor vascular responses in DOCA-salt hypertension.

Sexual dimorphism in vascular reactivity to ET-1 was also reported in SHR [106] and in normotensive Yucatan miniature pigs [107]. In SHRs, arteries from males exhibit greater sensitivity to ET-1, whereas no changes in sensitivity are observed in arterioles from females [106]. Interestingly, in Yucatan pigs, skeletal muscle arteries from females develop greater contractile force in response to ET-1 than arteries from males [107]. Exercise training increased ET-1-induced contractions in arteries from males, but not in arteries from females [107]. In coronary arteries from these pigs, exercise training reduces vascular sensitivity to ET-1, with the adaptation being greater in male than in female animals [108].

Sex differences were also observed in the pressor effects of ET-1. In anaesthetized rats, ET-1 produces rapid and transient falls in arterial BP and hindquarter resistance, followed by long-lasting increases in BP and hindquarter resistance. The initial ET-1-induced hypotension and vasodilation are similar in male and female rats; however, the pressor and hindquarter vasoconstrictor effects are significantly higher in male than female rats [109].

Although 17β-oestradiol attenuates ET-1-induced coronary artery constriction both in vitro and in vivo [110–112], testosterone significantly potentiates responses elicited by ET-1 [112]. Testosterone effects are not inhibited by the androgen receptor antagonists flutamide or cyproterone acetate and have a rapid time course, indicating that testosterone effects are not mediated by classical genomic activities [112].

Sex-related differences in other effects of ET-1, such as proliferative responses, have also been reported. ET-1 inhibits proliferation of smooth muscle cells derived from pig coronary arteries in female, but not in male,
In a rodent model of brain ischaemic injury, injection of ET-1 adjacent to the middle cerebral artery induces brain injury, with males having greater damage than females [114].

Furthermore, renal ET-1 expression and markers of renal injury, such as tubular dilatation, fibrinoid-like necrosis in glomeruli, interstitial cell infiltration, thickening of small arteries and collagen deposition, are all enhanced in male hypertensive rats compared with females [115,116]. Ovariectomy exacerbates renal injuries in DOCA-salt rats in a BP-independent fashion and also increases renal pre-pro-ET-1 mRNA expression, whereas hormonal treatment with oestrogen and progesterone or progesterone alone attenuates renal damage and ET-1 expression in ovariectomized DOCA-salt rats [115].

Female rats also display relative protection against other renal pathologies associated with increased ET-1 expression, such as postischaemic renal failure [117]. Greater survival rate, better renal and systemic haemodynamics and decreased renal pre-pro-ET-1 mRNA expression are observed in female rats compared with males. Orchidectomy of mature rats or sexual immaturity improves the rate of survival. Oestriadiol treatment of mature male animals also results in a significantly better survival, whereas ovariectomy, sexual immaturity or testosterone treatment have no impact on the course of renal failure in females [117].

**ET receptors**

Sex differences in ET-1 receptor density, as well as in the ratio of ET-1 receptor subtypes, favouring vasodilator effects in women, have been described. In human saphenous veins, men exhibit an increased total number of ET-1 receptors as well as an increased ratio of ETA to ETB receptors compared with women [102]. In DOCA-salt rats, vascular mRNA expression of ETB receptors is increased in males compared with females [87,88].

Experimental evidence indicates that ovarian hormones modulate ET receptor, mainly ETB receptor, expression. Endogenous fluctuations in oestrogen influence the affinity of ET-1 receptors in coronary arterial smooth muscle from female pigs [118]. 17β-Oestradiol induces down-regulation of ETB receptor mRNA in isolated rat cardiomyocytes [119]. Ovariectomy leads to a significant up-regulation of the ETB receptor mRNA and protein in the ventricular myocardium of SHR rats [119] as well as in vessels of DOCA-salt rats [87].

Recently, Kawanishi and co-workers [120] investigated the role of ETB receptors in sex differences in the development of DOCA-salt-induced hypertension by using the sl (spotting-lethal) rat, which carries a naturally occurring deletion in the ETB receptor gene. In wild-type rats, systolic BP elevation in response to DOCA-salt treatment was significantly lower in females compared with males. Sex differences are partially attenuated in ovariectomized animals [120]. In homozygous (sl/sl) rats, systolic BP in male, intact female and ovariectomized rats was markedly elevated by DOCA-salt treatment to the same extent, indicating that the sex-related differences in DOCA-salt-induced hypertension are abolished by the genetic ETB receptor deficiency [120]. In addition, vascular ET-1 production in male and ovariectomized sl/sl rats is much higher than that in intact homozygous females [120].

Male rats lacking functional ETB receptors (sl/sl) also have significantly higher systolic BP and plasma ET-1 levels, compared with heterozygous (sl/+) rats, on a normal salt diet. In contrast, sl/sl females given a normal salt diet have systolic BP similar to sl/+ control rats, yet with higher plasma ET-1 levels [121]. It appears that, although the higher plasma ET-1 levels in male sl/sl rats produce higher systolic BP, female rats exhibit a protective mechanism, i.e. females display normal BP despite elevated plasma ET-1 levels.

**Responses to ET receptor antagonists**

Male DOCA-salt animals exhibit a marked and greater BP-lowering effect in response to bosentan, a mixed ETA/ETB antagonist, compared with female DOCA-salt rats, supporting further the suggestion that differential activation of ET-1 pathways play a role in the higher BP levels observed in male DOCA-salt-hypertensive rats [105]. Removal of the ovaries and, consequently, a significant reduction in oestradiol and progesterone levels, exacerbates the BP-lowering effect of bosentan, whereas oestradiol or oestradiol plus progesterone restores plasma levels of the hormones and effects of bosentan [87]. Oral administration of ABT-627, an ETA receptor antagonist, to sl rats for 2 weeks also reduced BP by a greater magnitude in intact female compared with male animals [120].

Sex differences in the ET system may also be associated with sex-related differences in response to antihypertensive drugs other than ET receptor antagonists. Obese female SHHF/Mcc-fa(cp) rats are resistant to the antihypertensive effects of irbesartan, an AT1 receptor antagonist, compared with female DOCA-salt rats, supporting further the suggestion that differential activation of ET-1 pathways play a role in the higher BP levels observed in male DOCA-salt-hypertensive rats [105]. Removal of the ovaries and, consequently, a significant reduction in oestradiol and progesterone levels, exacerbates the BP-lowering effect of bosentan, whereas oestradiol or oestradiol plus progesterone restores plasma levels of the hormones and effects of bosentan [87]. Oral administration of ABT-627, an ETA receptor antagonist, to sl rats for 2 weeks also reduced BP by a greater magnitude in intact female compared with male animals [120].

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**CONCLUSIONS AND PERSPECTIVES**

Hypertension-related end-organ injury is one of the most common causes of mortality affecting both men...
and women in industrialized nations. Sex-related differences in renal and cardiovascular function as well as in arterial hypertension are well established. Although gonadal hormones seem to play a major role in sex-related differences in cardiovascular and renal (patho)physiological processes, the precise mechanisms underlying their specific effects are poorly understood.

Sex-related differences in the ET system as well as modulatory actions of the gonadal hormones on the ET pathway are evident. Both gene regulation at the transcriptional level and ECE activity contribute to sex differences in ET-1 production, with females displaying lower plasma and tissue ET-1 levels than males. Males also exhibit greater ET-1-mediated vasoconstrictor, pressor, mitogenic and inflammatory responses in comparison with females, which may be associated with an increased number or increased function of smooth muscle ETA and ETB receptors in males and/or greater activation of endothelial cell (or clearance) ETB receptors in females.

Although it is increasingly evident that the ET pathway plays a different role in cardiovascular and renal (patho)physiological processes in males and females, many aspects await for further investigation. For example, it has not yet been demonstrated whether oestrogen-responsive or androgen-responsive elements are present or, more importantly, if they are operational in the promoter of genes that constitute the ET-1 system: pre-pro-ET-1, ECE-1, ECE-2, chymase, ETA and ETB receptors. Although gonadal hormones may directly modulate the expression of components of the ET-1 system through activation of hormone-responsive elements, they may also interfere with the activity of transcription factors that are essential for each one of these promoters. In addition, sex and/or gonadal hormones may affect the expression and activity of specific messengers in the signalling pathways activated by ET-1, including several isoforms of PKC, members of the MAPK family, Pyk2, PI3K, JAK2/STAT and NADPH oxidase/ROS generation. Accordingly, very scarce information is available on these topics and the ET system and ET-1-activated signalling pathways constitute a fertile ground to investigate mechanisms of sex-related differences in cardiovascular and renal (patho)physiological processes.

Sex-associated differences in the number and function of ETB receptors seem to be particularly important in the specific characteristics of hypertension between females and males, at least in salt-sensitive models of hypertension. Because ET-1 and ETA/ETB receptors are expressed in almost every cell, it would be interesting to evaluate the existence, or not, of sex-related differences in the ET pathway in other organs/systems involved in BP control. Furthermore, since ovarian hormones modulate the expression and function of ETB receptors and because the effects of 17β-oestradiol can be modified by the presence or simultaneous administration of progesterone, it would be interesting to address the modulatory effects of all (not only 17β-oestradiol) gonadal hormones on the ET-1 system.

With further insights into the sex differences in the mechanisms that control the renal and cardiovascular function, we may learn that men and women could require different antihypertensive regimens. Although the inclusion of women in clinical trials may help to elucidate this aspect, ensuring that sex differences in cardiovascular and renal (patho)physiological processes are brought to the foreground will make sound evidence.

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Endothelin, sex and hypertension


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