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Vascular Endothelin Receptor Type B: Structure, Function and Dysregulation in Vascular Disease

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Abstract

Endothelin-1 (ET-1) is a major regulator of vascular function, acting via both endothelin receptor type A (ET_AR) and type B (ET_BR). Although the role of ET_AR in vascular smooth muscle (VSM) contraction has been studied, little is known about ET_BR. ET_BR is a G-protein coupled receptor with a molecular mass of ~50 kDa and 442 amino acids arranged in seven transmembrane domains. Alternative splice variants of ET_BR and heterodimerization and cross-talk with ET_AR may affect the receptor function. ET_BR has been identified in numerous blood vessels with substantial effects in the systemic, renal, pulmonary, coronary and cerebral circulation. ET_BR in the endothelium mediates the release of relaxing factors such as nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor, and could also play a role in ET-1 clearance. ET_BR in VSM mediates increases in [Ca²⁺]_i, protein kinase C, mitogen-activated protein kinase and other pathways of VSM contraction and cell growth. ET-1/ET_AR signaling has been associated with salt-sensitive hypertension (HTN) and pulmonary arterial hypertension (PAH), and ET_AR antagonists have shown some benefits in these conditions. In search for other pathogenetic factors and more effective approaches, the role of alterations in endothelial ET_BR and VSM ET_BR in vascular dysfunction, and the potential benefits of modulators of ET_BR in treatment of HTN and PAH are being examined. Combined ET_AR/ET_BR antagonists could be more efficacious in the management of conditions involving upregulation of ET_AR and ET_BR in VSM. Combined ET_AR antagonist with ET_BR agonist may need to be evaluated in conditions associated with decreased endothelial ET_BR expression/activity.

Keywords

endothelium; smooth muscle; calcium; blood pressure; hypertension

INTRODUCTION

Endothelin is an endothelium-derived contracting factor and a potent vasoconstrictor [1]. Since the discovery of endothelin-1 (ET-1), great advances have been made in our understanding of the various endothelins, their production, plasma levels and clearance. Further research has enhanced our knowledge of the different endothelin receptors and their tissue and cellular distribution, signaling pathways, role in the regulation of vascular function, and dysregulation in cardiovascular disease (CVD).

The vascular effects of ET-1 are mediated by at least two receptor subtypes, endothelin receptor type A (ET_AR) and type B (ET_BR) [2]. Several studies have provided detailed

information on ET_AR, its role in vascular smooth muscle (VSM) contraction and cell growth, and its pathological role in hypertension (HTN) and other CVD [3, 4]. However, less is known about the vascular ET_BR and its potential dysregulation in CVD. This commentary will discuss reports published in the PubMed database to provide insights into the role of ET_BR in the regulation of vascular function and blood pressure (BP), and how alterations in its expression and signaling mechanisms could lead to HTN and other CVD. The review will briefly describe the various ET peptides, their plasma and vascular tissue levels and the pathways involved in their synthesis and metabolism. We will then address ET_BR structure, subtypes, vascular tissue distribution, signaling pathways, and agonists/antagonists. Finally, we will discuss the pathological changes in vascular ET_BR in HTN and other CVD and the potential use of ET_BR modulators in the management of vascular disease.

Endothelin Synthesis, Metabolism and Clearance

Endothelins include the 21-aa peptides ET-1, -2 and -3, which share structure homology with the snake venom sarafotoxin b and c (S6b, S6c). ET synthesis is initiated by preproET gene transcription and the production of long 203-aa preproETs, which are cleaved by furin-like protease to biologically inactive 37- to 41-aa big ETs. Big ETs are cleaved by ET converting enzymes (ECEs), members of the metalloprotease family, to produce active ET peptides [4]. In endothelial cells (ECs), ET-1 is stored in Weibel-Palade bodies [5]. Upon EC activation by chemical stimuli or mechanical shear stress, which increase cytosolic Ca²⁺ influx [5], these storage granules relocate from the cytoplasm to the plasma membrane and release ET-1 by exocytosis [6]. ET-1 then acts in an autocrine or paracrine fashion on ET receptors in ECs and VSMCs [4]. ET-1 is also produced by airway epithelial cells, cardiomyocytes, fibroblasts, leukocytes and mesangial cells [7]. ET synthesis is regulated mainly at the gene transcription level. PreproET-1 mRNA is upregulated during cardiovascular stress and in response to vasoactive agents such as angiotensin II (AngII) and cytokines such as tumor necrosis factor- α and transforming growth factor- β [3, 7]. Hypoxia is a major stimulus of ET-1 synthesis and signaling [8] [9], and normobaric hypoxia stimulates preproET-1 gene expression in the rat [10]. The hypoxia-induced upregulation of ET-1 could play a role in pulmonary arterial hypertension (PAH), and the placental ischemia/hypoxia associated with preeclampsia. In ECs, preproET-1 mRNA expression increases then decreases during shear stress, and is downregulated by nitric oxide (NO), prostacyclin (PGI₂) and atrial natriuretic factor [7]. ECE-1 expression, and consequently ET-1 synthesis, is also regulated by protein kinase C (PKC), ets-1 transcription factor and cytokines.

The plasma levels of ET-1 are very low; 0.7 to 5 pg/mL in healthy human adults and 0.7 to 4.9 fmol/mL in Wistar rats. Tissue levels of ET-1 are also low, reaching only 120 pg/g in rat aorta. The low plasma levels of ET could be due to its continuous metabolism and clearance. In normal adults, the 24 hr urinary ET excretion is 1.7 pg/mL to 6.8 ng/mL [7]. ET clearance from the circulation is achieved by endothelial ET_BRs, also termed “clearance receptors” (Fig. 1) [11] and in the lung by endocytosis and degradation [12]. Following intravenous injection, ET-1 is rapidly removed from the circulation and retained primarily in the lungs, kidney and liver, and this effect is inhibited by ET_BR, but not ET_AR antagonists [13]. In support of ET_BR-mediated clearance of ET-1, plasma ET-1 levels are increased during ET_BR blockade and in transgenic ET_BR-deficient rats [14]. The lungs contain the highest ET_BR density as it has ~50% of the endothelium of the entire vascular tree. Considering the high blood volume in the lungs, the pulmonary endothelium acts as an efficient clearing mechanism, retaining 60% of circulating ET-1 at each pulmonary pass [12]. ET-1 clearance also takes place in the liver and kidney, and bilateral nephrectomy in rats impairs removal of exogenous ET-1 [15].

ET_BR Structure, Regulatory Domains and Active Sites

The vascular effects of ET are mediated by ET_AR and ET_BR [2]. A third receptor subtype with high selectivity for ET-3, termed ET_CR, was characterized in the amphibian *Xenopus laevis*. ET_AR structure and function have been extensively characterized [3, 7], but less is known about ET_BR. The human ET_BR gene is located on chromosome 13 and has 7 exons and 6 introns. The ET_BR is 442 aa long with a predicted molecular mass ~50kDa and 64% sequence homology with ET_AR [7]. ET_BR in mouse, rat, bovine, goat and pig is 441–443 aa long and displays a high degree of sequence homology with human ET_BR (97% for canine, 90% for pig, 88% for mouse/rat/bovine) [16].

ET_BR belongs to the rhodopsin-type superfamily of G-protein coupled receptors (GPCR) consisting of a long extracellular N-terminus sequence, seven helical transmembrane domains (TMDs), 3 extracellular and 3 intracellular loops, and a cytoplasmic C-terminus tail (Fig. 1). TMD I–III and VII and intervening extracellular loops constitute agonist binding. TMD IV–VI and the adjacent extracellular loops are involved in receptor binding selectivity, and the carboxyl terminal tail constitutes the signaling domain [17]. Post-translational modification of ET_BR includes palmitoylation, phosphorylation and possibly glycosylation [18]. Site directed mutagenesis and [³H] palmitic acid studies revealed three cysteine residues (Cys402, 403 and 405) in the carboxyl tail of human ET_BR as potential palmitoylation sites [19]. The degree of C-terminal palmitoylation may affect coupling with G-proteins, the downstream signaling pathways, and the ET_BR phosphorylation pattern. Studies have identified 13 phosphorylation sites [18], mainly at the end of the C-terminus (435–442 aa) (Fig. 1). ET_BR phosphorylation may affect receptor function, and phosphorylation in the third cytoplasmic loop and/or C-terminal tail by GPCR kinases (GRKs) or β -arrestins may be involved in receptor desensitization [20]. ET_BR contains a glycosylation site at Asn59, but does not appear to affect ligand binding.

The canonical ET_BR was cloned from human liver and placental cDNA libraries. Several alternative splice variants of human ET_BR have been identified. The first splice variant has an additional 10 aa at the second cytoplasmic loop of ET_BR, but has similar binding and functional characteristics as wild-type ET_BR [21]. A second splice variant from human placental libraries has 436 aa residues and differs from wild-type ET_BR in the last 52 aa of the carboxyl terminal. Although this splice variant has the same binding properties as wild-type ET_BR, it lacks functional coupling to phosphoinositide turnover [22]. A third splice variant in human melanoma cell line has its transcription initiation 939bp upstream from wild-type ET_BR, an additional ~90 aa at the N-terminus and a shorter 3' untranslated region, but exhibits similar binding and functional characteristics as wild-type ET_BR [23].

ET_BR Binding to ET-1 and Receptor Trafficking

ET receptors bind ET-1 with high affinity. ET-1 binds polyvalently to ET_AR and both ET_AR antagonists and endogenous physiological antagonists allosterically reduce ET_AR function. In rat mesenteric resistance arteries, ET-1 binds tightly to ET_AR and causes long-lasting contraction (> 20 min) that is only partly and reversibly relaxed by the ET_AR antagonists BQ123 and SB234551. Because of the tight binding of ET-1 to ET_AR, research has been directed to the agonist residence time on the receptor and the rate of dissociation of the agonist-receptor complex. The neuropeptide calcitonin-gene-related peptide and its receptor can act as a physiological endogenous indirect negative allosteric modulator of ET_AR, suggesting that cross-talk between peptidergic GPCRs could reduce the affinity and increase the rate of dissociation of the ET-1/ET_AR complex [24].

ET_BR differs from ET_AR in its binding affinity to ET-1, and this may ultimately affect the fate of the receptor and ET-1. Both ET_AR and ET_BR are rapidly internalized upon agonist

stimulation, a process that involves GRK, arrestin, dynamin and clathrin. Internalized ET_AR and ET_BR are directed to Rab5 positive early endosomes (sorting endosomes), then targeted to different intracellular fates. ET_AR is directed to the pericentriolar compartment for recycling, and subsequently reappears back at the plasma membrane in a ligand-unbound state [25]. Ligand-bound ET_BR is internalized, but, unlike ET_AR, it is sorted to the late endosomes/lysosomes for degradation; a major pathway for ET-1 “clearance” [26]. Bioinformatics and mutation analyses suggest that recycling of ET_AR is mediated by 390–406 aa within the internal postsynaptic density 95/disc-large/zona (PDZ) ligand-binding peptide motif in the C-terminal tail. This motif is lacking in the C-terminal region of ET_BR, providing a mechanism for the divergent endocytotic sorting of ET_AR vs. ET_BR [27]. Studies have shown that ET_BRs are more readily accessible to circulating ET-1 than ET_ARs likely because ET_BRs outnumber ET_ARs or due to the close proximity of the ET_BR pool on ECs, resulting in greater changes in circulating ET-1 levels during blockade of ET_BR as compared to ET_AR [28].

ET_AR and ET_BR Dimerization

ET_AR and ET_BR may form homodimers or heterodimers or higher order oligomers that may modify ligand binding, receptor activation, desensitization and transmembrane signaling. ET_AR and ET_BR traffic to the cell surface first as monomers then as constitutive heterodimers. Formation of ET_BR/ET_AR heterodimers is supported by the observation that ET-1 is a bivalent ligand that can bridge between the two receptor subtypes in the plasma membrane [29]. ET_BR internalizes more slowly when present as ET_AR/ET_BR heterodimer, and the heterodimer dissociates after prolonged exposure to ET_BR agonist and subsequent internalization and endocytosis, whereas homodimers are resistant to ligand-induced dissociation [29]. Both selective ET_AR and ET_BR antagonists may be required to inhibit the function of heterodimeric receptor. Cross-talk between ET_AR and ET_BR has been suggested [30]. Binding studies have shown that ET_AR and ET_BR activation of intracellular signaling pathways may influence ET-1 binding to its receptor subtypes. However, whether these binding properties would translate into functional ET_AR/ET_BR cross-talk has not been fully tested [31]. In an *in situ* cranial window of the rat basilar artery, ET-1 caused contraction that was relaxed by 50% by the ET_AR antagonist BQ123, and the remaining 50% of ET-1 contraction was relaxed by the ET_BR antagonist BQ788, suggesting an ET_BR-mediated component of contraction. BQ788 in the absence of BQ123 did not cause relaxation, and subsequent addition of BQ123 completely relaxed the ET-1 contraction. Also, PD145065, a mixed ET_AR/ET_BR antagonist, completely relaxed ET-1 contraction both in the absence and presence of BQ123. The ability of ET_AR antagonist to completely relax ET-1 contraction only in the presence of ET_BR antagonist, and vice-a-versa, suggests cross-talk between ET_AR and ET_BR in the cerebral circulation [32]. ET_BR may form heterodimers with other receptors such as the dopamine D3 receptor and angiotensin type 1 receptor (AT₁R) in the renal proximal tubules [28] [33] [34] [35], and the receptor heterodimerization may play a role in salt and water homeostasis and BP control.

ET_BR Tissue and Subcellular Distribution

ET_BR predominates in ECs and is present in low densities in VSMCs of some vascular beds such as the aorta, coronary arteries, mesenteric arteries and veins of experimental animals and in human mammary artery (Table 1) [7]. ET_BR is also expressed in vascular adventitial fibroblasts [36], the myocardium, renal tubules, airway smooth muscle, hepatocytes, osteoblasts, central and peripheral neurons, endocrine tissues and reproductive tract. ET_AR is expressed in VSMCs, vascular adventitial fibroblasts [36], airway smooth muscle, cardiomyocytes, liver stellate cells, neurons, osteoblasts, melanocytes, keratinocytes, adipocytes and the reproductive system [7].

Studies have examined the subcellular distribution of ET_AR and ET_BR in various cardiovascular cell types including human cardiac and endocardial cells, and aortic VSMCs and ECs. Both ET_AR and ET_BR appear in the plasma membrane, cytosol and the nucleus including the nuclear envelope membrane, but only ET_BR appears in the nucleoplasm [37] (Table 1). Whether the differences in the cellular and subcellular distribution of ET receptor subtypes play a role in the different efficacy of ET receptor antagonists in pulmonary arterial disease as compared to other systemic vascular and cardiac disease remains to be examined.

Endothelial ET_BR Signaling. NO, PGI₂, EDHF

Depending on the effector cell type, two ET_BR subtypes have been suggested, vasodilator ET_BR in ECs and vasoconstrictor ET_BR in VSMCs [38]. In ECs, ET_BR is coupled to signaling pathways that increase the release of relaxing factors such as NO (Fig. 2). In rat aorta, the ET_BR agonist IRL-1620 binds to endothelial ET_BR and causes an increase in [Ca²⁺]_i which activates NO synthase to produce NO. NO diffuses into VSMCs, where it stimulates soluble guanylate cyclase to produce cGMP and induce vascular relaxation. NO and NO donors inhibit ET-1 release or counteract its vasoconstrictor effects in VSM, and *in vivo* administration of the NOS inhibitor N^ω-nitro-L-arginine methyl ester (L-NAME) enhances ET-1 release from ECs. ET_BR mediates the release of other endothelium-derived vasodilators such as PGI₂ and endothelium-derived hyperpolarizing factor (EDHF) [7] (Fig. 2). In endothelium-intact rat carotid artery, IRL-1620 causes relaxation that is not abolished by L-NAME or the cyclooxygenase (COX) inhibitor indomethacin, but abolished by tetraethylammonium, a nonselective K⁺ channel blocker, supporting an EDHF component of ET_BR-mediated relaxation. Also, 4-aminopyridine, but not apamin, charybdotoxin or glibenclamide reduces IRL-1620 induced relaxation, indicating that voltage-dependent K⁺ channel (K_v) but not small-conductance Ca²⁺-activated K⁺ channel (K_{Ca2.3}), intermediate-conductance Ca²⁺-activated K⁺ channel (K_{Ca3.1}) or ATP-sensitive K⁺ channel (K_{ATP}) contributes to the ET_BR/EDHF-mediated response [39].

VSM ET_BR Signaling. Ca²⁺, PKC, Rho-Kinase, MAP Kinase

The vasoconstrictor effects of ET-1 are largely mediated by ET_AR. ET_AR is coupled to G_q/G₁₁, activation of phospholipase C-β (PLC-β) and breakdown of phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG) (Fig. 2). IP₃ causes transient Ca²⁺ release from the sarcoplasmic reticulum. ET-1 also stimulates influx of extracellular Ca²⁺ through dihydropyridine-sensitive voltage-gated Ca²⁺ channels in VSMCs [40]. Similar to ET_AR, VSM ET_BR mediates changes in intracellular free Ca²⁺ concentration ([Ca²⁺]_i). In preglomerular VSMCs, IRL-1620 causes a rapid increase in [Ca²⁺]_i that amounts to ~1/3 of ET-1 induced [Ca²⁺]_i [41]. Likewise, in isolated rat VSMCs of small mesenteric arteries, the ET_BR agonist S6c increases [Ca²⁺]_i to ~1/2 the ET-1 [Ca²⁺]_i response [3]. In rabbit pulmonary artery VSMCs, ET-1 induces a transient increase in [Ca²⁺]_i that is abolished by ryanodine and thapsigargin, suggesting a role of Ca²⁺ release from ryanodine- and IP₃-sensitive Ca²⁺ stores. The transient ET-1 induced [Ca²⁺]_i is also inhibited by the ET_AR antagonist BQ123 and ET_BR antagonist BQ788, suggesting a role for both receptors in Ca²⁺ signaling. In the same VSMCs, IRL-1620 induces a transient followed by sustained increase in [Ca²⁺]_i and an increase in Ca²⁺ current that is blocked by nifedipine, suggesting a role of voltage-gated Ca²⁺ channels [42]. The ET_AR or ET_BR-mediated increase in Ca²⁺ binds calmodulin to form a complex, which activates myosin light chain (MLC) kinase and leads to MLC phosphorylation, actin-myosin interaction and VSM contraction [7].

ET_AR and ET_BR may activate other types of Ca²⁺ channels and other mechanisms of VSM contraction. When compared with ET-1 and ET-2, ET-3 causes little change in [Ca²⁺]_i in rat preglomerular VSMCs. Also, while ET-1 and S6c decrease renal blood flow, in

preglomerular VSMCs ET-1 produces a typical biphasic $[Ca^{2+}]_i$ response that is not abolished by diltiazem, while S6c has no effect [43], suggesting that ET-1 induced Ca^{2+} influx involves channels other than voltage-gated Ca^{2+} channels such as receptor-operated (ROCCs) and store-operated Ca^{2+} channels (SOCCs) and that ET_B R-mediated preglomerular constriction may involve a Ca^{2+} -independent mechanism. ET-1 induced VSM contraction and MLC phosphorylation could involve ET_A R or ET_B R-mediated G_{12} -coupled activation of reverse mode Na^+/Ca^{2+} , and G_{13} -coupled Rho-kinase dependent inhibition of MLC phosphatase. Also, ET_A R/ ET_B R-mediated increase in DAG stimulates PKC [7], and ET_B R may mediate inhibition of MLC phosphatase via PKC-dependent phosphorylation of CPI-17 [44]. In rat cerebral artery, the PKC inhibitor Ro-31-8220 attenuates S6c-induced contraction and ET_B R expression [45]. Mitogen-activated protein kinase (MAPK) is an important mediator of ET_B R transcription. In rat mesenteric artery in organ culture, the Raf inhibitor SB386023b, acting upstream from MEK/ERK $_{1/2}$, and the MEK inhibitor PD98059 cause a decrease in ET_B R mRNA levels [46]. Also, in porcine coronary artery in organ culture, S6c causes contraction and increases ET_B R mRNA and protein levels that are inhibited by the PKC inhibitor bisindolylmaleimide or Ro-32-0432, the MEK inhibitor PD98059, and the C-jun terminal kinase (JNK) inhibitor SP600125, supporting that PKC and MAPK are involved in the regulation of ET_B R expression/activity [47]. Also, Ca^{2+} /calmodulin-dependent protein kinase II (CAMK-II) may interact with the ERK $_{1/2}$ pathway to enhance ET_B R expression in rat cerebral artery. In human VSMCs, the ET_A R antagonist BQ123, but not ET_B R antagonist BQ788, inhibits ET-1 induced activation of ERK $_{1/2}$, and S6c causes only slight and transient activation of ERK $_{1/2}$, suggesting a predominant role of ET_A R, and highlighting the diverse signaling cascades in different vascular beds from different species. Other ET_A R/ ET_B R-mediated signaling pathways include phospholipase D with generation of DAG, phospholipase A $_2$ with release of arachidonic acid, the Na^+/H^+ exchanger, Src-family tyrosine kinases, phosphatidylinositol 3-kinase (PI $_3$ K) and p38 MAPK [3]. For instance, in Chinese hamster ovary (CHO) cells expressing recombinant human ET_B R, ET-1 causes sustained $[Ca^{2+}]_i$ influx that involves $G_{(q/11)}$ /PLC-independent, but p38 MAPK-dependent activation of the Na^+/H^+ exchanger [48].

ET_B R Agonists and Antagonists

ET_A R has subnanomolar affinity for ET-1 and ET-2 and lower affinity for ET-3 ($ET-1=ET-2 \gg ET-3$) (Fig. 3). ET_B R binds with the same affinity for $ET-1=ET-2=ET-3$ as well as S6b and S6c [7]. S6b does not discriminate between ET_A R and ET_B R, but S6c is highly selective for ET_B R. Other ET_B R agonists include the synthetic linear analogs BQ3020 and IRL-1620. [Ala $_{1,3,11,15}$]ET-1 is a linear analog of ET-1 in which the disulfide bridges are removed by substitution of Ala for Cys residues, and is a selective ET_B R agonist. IRL-1620 is highly specific as compared with S6c, which may stimulate receptors other than ET_B R [38]. ET_B R antagonists include BQ788, IRL-1038, IRL-2500 and RES-701. Non-peptide ET_B R antagonists include A-192621 and Ro46-8443 (Table 1).

ET_A R- ET_B R Balance

Under physiological conditions, the vasodilator effects of endothelial ET_B R oppose the vasoconstrictor and mitogenic effects of ET_A R and possibly ET_B R in VSMCs, thus creating a fine balance between ET_A R- and ET_B R-mediated effects in the circulation. In pathological conditions, there could be not only upregulation of ET_A R, but also downregulation of endothelial ET_B R and its vasodilator effects thus promoting the vasoconstrictor and mitogenic effects of ET-1 in VSM via activation of ET_A R and possibly ET_B R. Also, because of the ET_B R clearance properties, disruption of functional ET_B R may reduce ET-1 clearance and increase its levels in the circulation, thus allowing more ET-1 to activate ET_A R, and resulting in further amplification of its vasoconstrictor and VSM hypertrophic effects. Studies with ET_B R transgenic and knockout (KO) animals as well as some clinical

studies have provided insights into the vascular protective role of ET_BR, and suggested that dysregulation of ET_BR could be associated with certain disorders such as Hirschsprung's disease and CVD such as HTN, coronary artery disease (CAD), cerebral ischemia and atherosclerosis.

ET_BR-Deficient Phenotypes

Mutations in EDNRB gene have been linked to Hirschsprung's disease in humans, a congenital disease characterized by aganglionic megacolon, absence of enteric ganglia and lack of innervation to the lower gastrointestinal tract. The disease is associated with polymorphism and several missense mutations in EDNRB gene that lead to decreased expression, changes in cell signaling, and loss of ET_BR function [49]. Splice variants of the EDNRB gene from patients with Hirschsprung's disease that are lacking 134 bp of exon 5 and others containing the substitution A950G in exon 4 of EDNRB have been associated with loss of ET_BR function. Other mutations of Hirschsprung's disease include F29L, G57S, A183G, W276C, A305N, R319W, M374I and P383L [49]. Mutation of Ser390 by L-Arg at the origin of the C-terminal tail of ET_BR reduces the capacity to increase $[Ca^{2+}]_i$ and adenylyl cyclase, but does not affect receptor binding [50]. Substitution of the highly conserved Asp147 by Ala at TMD II and Try276 by Cys in TMD V affects G_q and PLC signaling, and reduces Ca^{2+} mobilization without affecting ligand binding [51] (Fig. 1). The human phenotype may also have mutations in the ET-3 gene [49] and Waardenburg Syndrome type 4A, a genetic disorder associated with deafness, neural crest and pigmentation anomalies traceable to at least six genes that include ET-3 and EDNRB genes, supporting the importance of ET-3/ET_BR signaling.

Certain mouse and rat strains such as piebald mice (*s*), piebald-lethal (*sl*) mice, and spotting-lethal (*sl*) rats exhibit striking similarities to the human condition, and have a naturally occurring mutation of EDNRB gene that produce a phenotype with aganglionic megacolon and developmental defects in neural crest cell migration and differentiation. Piebald (*s*) mice exhibit a natural spontaneous mutation in EDNRB gene with a 5.5-kb retroposon-like element in intron 1 that causes premature termination and aberrant splicing of ~75% of the normally initiated EDNRB transcript, resulting in ~25% reduced EDNRB levels and partial loss-of-function [52]. These mice exhibit reduced white spotting of their coat by ~20% but do not manifest megacolon. Piebald-lethal (*sl*) mice exhibit a natural deletion mutation of the entire EDNRB gene, identical to that of homozygous EDNRB^{-/-} KO mouse, resulting in a phenotype of white-spotted coat, aganglionic megacolon and juvenile death at 2–4 week of age [53]. Heterozygous ET_BR-deficient mice have been generated by crossing inbred mice heterozygous for targeted disruption of the EDNRB gene with mice homozygous for the piebald (*s*) mutation of the EDNRB gene. Heterozygous ET_BR^{+/-} mice display increased BP that can be reversed by the ET_AR antagonist BQ123 but not the ET_BR antagonist BQ788, and elevated plasma ET-1 levels likely due to impaired clearance via ET_BR [54]. Other groups have produced a viable ET_BR-deficient mouse by genetic rescue of the lethal ET_BR KO mice by breeding with mice harboring a dopamine-β-hydroxylase ET_BR transgene in the enteric nervous system. The 'rescued' ET_BR-deficient mice do not have megacolon, but develop salt-sensitive HTN, and impaired endothelial function independent of high salt (HS) diet or HTN, suggesting that in this model salt-sensitive HTN is not mediated by endothelial dysfunction [55]. Interestingly, EC-specific ET_BR KO mice, generated by using a *Cre-loxP* approach, have aortic endothelial dysfunction, decreased release of NO and increased plasma ET-1 levels, suggesting that endothelial ET_BR mediates a tonic vasodilator effect and possibly clearance of ET-1. However, in contrast to models of total ET_BR ablation, EC-specific ET_BR KO mice do not develop salt-sensitive HTN, suggesting that non-EC ET_BR is important for BP regulation [56].

Spotting lethal (*sl*) rats have a naturally occurring mutant EDNRB gene with a 301-bp deletion between exon 1 and intron 1 including the last 265 bp from exon 1, leading to aberrant splicing of mRNA, missing coding sequence for TMD-I and -II of ET_BR, and juvenile death. Transgenic rescue of the intestinal phenotype in the (*sl*) rat has been developed by harboring a wild-type rat ET_BR cDNA, whose expression is driven by the human dopamine- β -hydroxylase promoter. Transgenic ET_BR-deficient rats do not express ET_BR driven by the endogenous promoter, but express dopamine- β -hydroxylase-driven ET_BR in adrenergic tissues such as the adrenal medulla and sympathetic ganglia and therefore exhibit normal enteric nervous system development and normal BP, but become severely hypertensive when placed on HS diet [14] (Table 2).

ET_BR and Regulation of the Systemic Circulation

ET_BR plays an important role in the systemic circulation. Infusion of ET-1 or IRL-1620 in Sprague-Dawley rat elicits a biphasic BP response consisting of a fast onset and transient depressor effect (10 to 45 sec) likely due to activation of endothelial ET_BR, followed by a prolonged pressor response (3 to 4 min) due to activation of ET_AR and possibly ET_BR in VSM (Table 3). The pressor response to IRL-1620 is much smaller than that of ET-1. ET-1 or IRL-1620 also increases total vascular resistance, reduces cardiac output, and causes mesenteric and renal vasoconstriction [57]. Administration of the ET_AR antagonist BQ123 or FR-139317 enhances the initial depressor effect of ET-1 and reduces the pressor effect, supporting ET_BR-mediated depressor effects. However, the pressor and regional constrictor effect of ET-1 are only inhibited by 25–50% even with high doses of ET_AR antagonists, suggesting a vasoconstrictor role for ET_BR [58]. In the conscious rat, administration of the ET_BR antagonist BQ788 does not reduce the pressor response induced by ET-1, but rather accelerates its onset and potentiates the increase in total vascular resistance, suggesting a predominant role of endothelial ET_BR in limiting the pressor effects of ET-1 [59]. On the other hand, the ET_BR agonist S6c is a potent pressor agent in the pithed rat [60], causing a transient decrease in BP followed by a long-lasting pressor response and marked renal and mesenteric vasoconstriction. Also, the ET_BR antagonist Ro-468443 causes reduction in BP in anaesthetized normotensive rats, providing *in vivo* evidence for VSM ET_BR-mediated vasoconstriction [61].

In the anesthetized rabbit, infusion of the ET_AR antagonist BQ123 markedly reduces the pressor response to both ET-1 and big ET-1. In contrast, infusion of the ET_BR antagonist BQ788 potentiates ET-1 or big ET-1-induced pressor effects, supporting that blockade of endothelial ET_BR-mediated vasodilation would enhance the systemic vasoconstrictor and pressor effects of ET-1 acting through ET_AR. BQ788 also increases the plasma levels of ET-1 and big ET-1, suggesting that ET_BR blockade could displace ET-1 from clearance ET_BR thus allowing more ET-1 to activate ET_AR [62].

In healthy men, 15 min infusion of BQ788 in the left antecubital vein had no effect on mean arterial pressure (MAP), but caused systemic vasoconstriction, suggesting a role of endothelial ET_BR in promoting systemic vasodilation likely through the release of NO and other vascular relaxing factors [63]. In contrast, infusion of BQ123 in the brachial artery for 60 min caused a dose-dependent decrease in systemic vascular resistance and MAP in healthy men [64], indicating that ET_AR-mediated vascular tone contributes to the maintenance of basal systemic vascular resistance and MAP, although ET_AR blockade with BQ123 could also shift ET-1 to activate endothelial ET_BR and promote further vasodilation.

Taken together these findings highlight the importance of endothelial ET_BR in reducing the systemic pressor effects of ET-1 and MAP partly by opposing the vasoconstrictor actions of ET_AR, and partly by enhancing ET-1 clearance from the circulation and suppressing the amount of ET-1 available for ET_AR to promote vasoconstriction.

Effects of ET_BR in Systemic Arteries and Veins

ET_BR mediates significant vascular effects ranging from vasodilation to vasoconstriction depending on the arterial or venous bed studied. *In vitro* studies on rat mesenteric arteries mounted on wire or perfusion myographs have shown that the ET_BR agonists ET-3 and S6c produce small constrictor effects [3] [4]. The expression and contractile effects of ET_BR appear to change in blood vessels in organ culture. In one study on freshly isolated rat mesenteric artery, ET-1 and ET-3 induced strong contractions, with ET-1 being 20-fold more potent likely due to ET_AR stimulation. In contrast, neither S6c nor IRL-1620 induced contraction likely because any VSM ET_BR-mediated vascular contraction was offset by endothelial ET_BR-mediated vascular relaxation. However, in mesenteric artery segments cultured for 24 hr, ET-3 is only 3-fold less potent than ET-1, and S6c and IRL-1620 induce contractions that are ~60% of ET-1 induced contraction, likely due to upregulation of VSM ET_BR. Removal of the endothelium has no effect on S6c-induced contraction, suggesting that the appearance of ET_BR-mediated contraction following organ culture may not involve downregulation of endothelial ET_BR or endothelium-derived vasodilators [65]. The upregulation of ET_BR expression and S6c-induced ET_BR-mediated contraction in organ culture may involve *de novo* transcription of the receptor, and activation of PKC, ERK_{1/2} MAPK and NF- κ B dependent mechanisms [66]. Other studies have shown that increased perfusion pressure in isolated rat mesenteric arteries results in increased ET_BR mRNA expression and protein [67], and periodic stretch increases ET_BR mRNA levels up to 10-fold in rat aortic VSMCs [68].

In human brachial artery, infusion of BQ123 caused forearm vasodilatation that was attenuated by 95% during an NO clamp by L-NMMA infusion, but not during inhibition of prostanoid generation. Co-infusion of BQ788 with BQ123 attenuated the vasodilatory response by 38%, supporting a role of endothelial ET_BR [69]. On the other hand, localized infusion of S6c causes constriction in human forearm resistance arteries and dorsal hand veins [70]. In human internal mammary artery precontracted with ET-1, both the ET_AR antagonist BQ123 and ET_BR antagonist BQ788 cause relaxation, suggesting a role of both ET_AR and VSM ET_BR in ET-1 induced contraction [71]. Other *in vitro* studies in human coronary artery and mammary artery have shown little vasoconstrictor effects of the ET_BR agonists S6c, BQ3020 and [Ala^{1,3,11,15}]-ET-1 [16].

Evidence also suggests a role of ET_BR in the venous circulation. Veins may have vasodilator ET_BR in ECs. In rat vena cava precontracted with PGF₂ α , S6c elicits marked relaxation both in the absence and presence of L-NAME, suggesting that both NO and EDHF pathways may be involved [72]. On the other hand, in human saphenous vein [73], rabbit saphenous vein [74], and rat mesenteric vein [75] and vena cava [76], S6c causes contraction suggesting VSM ET_BR-mediated venoconstriction. Thus, stimulation of ET_BR in the endothelium or VSM of systemic arteries and veins can cause vasodilator or vasoconstrictor effects, respectively, and dysregulation of ET_BR in different cell types and vascular beds may play a role in vascular disease.

Dysregulation of ET-1 and Vascular ET_BR in Hypertension

The prominent and prolonged vasoconstrictor effects of ET-1 have suggested a role in HTN. However, most patients with HTN show normal or slightly increased ET-1 levels [77]. Among African-Americans, plasma ET levels are greater in hypertensive than normotensive controls, but among individuals with similar severity of HTN plasma ET levels are not higher in African-Americans than Caucasians [77]. Upregulation of the ET system is commonly observed in severe cases of HTN associated with heart failure, CAD, atherosclerosis and PAH. For example, plasma levels of ET-1 and big ET-1 are markedly elevated in patients with heart failure compared with control subjects [78]. The discrepancy

in plasma ET-1 levels among hypertensive patients may be related to its rapid clearance from the blood stream. Also, ET-1 is mainly secreted in a polarized fashion from ECs to the underlying VSMCs, leading to minimal increases in circulating plasma ET-1. ET-1 tissue expression is increased in some forms of human HTN including salt-sensitive HTN, low renin HTN, and obesity and insulin resistance-related HTN [77]. Also, the 24 hr urinary excretion of ET-1 is greater in hypertensive patients with heart failure (17.0 ng/g urinary creatinine, UC) than control subjects (1.7 ng/g UC) [7].

ET-1 may also play a role in animal models of HTN such as deoxycorticosterone acetate (DOCA)-salt hypertensive rat [28] [77]. ET-1 levels are greater in the aorta of DOCA-salt hypertensive rats (730 pg/g) than control rats (120 pg/g) [79]. ET-1 levels are also increased in the vascular wall of DOCA-salt-treated spontaneously hypertensive rats (SHR), salt-loaded stroke-prone SHR, Dahl salt-sensitive rats, AngII-infused rats and 1-kidney 1-clip Goldblatt hypertensive rats, but not in SHR, 2-kidney 1-clip hypertensive rats or L-NAME-treated rats [77].

Decreased endothelial ET_BR may play a role in HTN. Chronic treatment of Sprague-Dawley rats with the ET_BR antagonist A-192621 is associated with increased BP, enhanced aortic contraction, and reduced endothelium-dependent aortic relaxation and NO production [80]. The vasoconstrictive effects of A-192621 are greater in rats on HS diet than normal salt diet, suggesting that endothelial ET_BR may influence basal vascular tone by activating the NO-cGMP pathway, a protective effect that is enhanced during HS diet [80]. Also, in hamsters, infusion of low dose of A-192621 did not cause pressor effects, but markedly reduced the transient hypotensive phase induced by intravenously injected ET_BR agonist IRL-1620. A-192621 alone or with the selective ET_AR antagonist atrasentan potentiated the pressor response to exogenous ET-1. Also, infusion of high dose of A-192621 for 16 days increased BP and plasma ET-1 levels. These findings are consistent with a role of endothelial ET_BR in the vasodilatory response to ET-1 and in clearance of endogenous ET-1, and therefore blockade of ET_BR potentiates ET_AR-mediated increase in vascular resistance and BP [81].

Role of ET_BR in Renal Circulation and Sodium and Water Homeostasis

ET_BR plays a role in the regulation of renal blood flow and the sodium and water balance. *In situ* studies in superfused kidney and afferent arterioles of Sprague-Dawley rats on HS diet (8%) showed increased relaxation to big ET-1, ET-1 and S6c, when compared to rats on normal salt diet (0.66%) [28]. Blockade of ET_BR with A-192621 abrogated the vasodilatory response to ET-1 during HS diet. Also, ET_BR expression in preglomerular microvessels was increased during HS, whereas ET_AR expression was unchanged, suggesting that the reduced vasoconstrictor response of afferent arterioles to ET peptides during HS diet is likely due to vasodilator actions of endothelial ET_BR [28].

ET_AR and ET_BR may contribute differently to the ET-1 response in afferent and efferent arterioles. In afferent arterioles, ET-1 induced similar constriction in ET_BR-deficient and wild-type mice, the ET_AR antagonist BQ123 inhibited this response in both groups, and the ET_BR agonists [Ala1,3,11,15]ET-1 and IRL-1620 had no effect on arteriolar diameter. In efferent arterioles, ET-1 caused stronger constriction in wild-type than ET_BR-deficient mice, BQ123 abolished constriction only in ET_BR-deficient mice, and [Ala1,3,11,15]ET-1 and IRL-1620 constricted only arterioles of wild-type mice. L-NAME decreased basal diameter in wild-type but not ET_BR-deficient mice, supporting contribution of ET_BR to basal NO release in efferent arterioles. Thus in afferent arterioles ET_AR mediates vasoconstriction and ET_BR has no vasoconstrictor or vasodilator action, while in efferent arterioles both ET_AR and ET_BR in VSM mediate vasoconstriction, and endothelial ET_BR mediates vasodilatation [82], highlighting the differences in ET_AR and ET_BR regulation in different renal arterioles.

The renal medullary ET-1 system and ET_BR play a role in the control of sodium and water excretion and BP [28] [83]. ET_BR is abundant in the epithelium of the collecting ducts of the renal medulla, and is considered the main inhibitory site of ET-1 action on sodium and water reabsorption. ET_BR activation increases renal medullary blood flow by increasing NO, vasodilator cyclooxygenase metabolites, and possibly cytochrome p-450 products such as 20-HETE [84], which sodium excretion.

In support of a role of ET_BR in sodium and fluid homeostasis, blockade of ET_BR or genetic ET_BR deficiency in rats or mice leads to salt-sensitive HTN [14, 80, 85]. Also, renal collecting duct-specific KO of ET_BR in mice is associated with HTN, supporting an antihypertensive role of ET_BR by enhancing sodium and water excretion [86]. Interestingly, the renal medullary production of 20-HETE, appears to involve ET_BR, and to be enhanced during HS diet. This is supported by the observation that blockade of ET_BR in the rat renal medulla during high dietary salt intake results in decreased renal medullary 20-HETE production and salt-sensitive HTN [84].

ET_BR may also be cardio- and renoprotective in HTN, and DOCA-salt-induced cardiovascular and renal dysfunction deteriorates following treatment with an ET_BR antagonist. The protective role of ET_BR against HTN may also be related to its effects on oxidative stress. Activation of clearance ET_BR could decrease the amount of ET-1 available to activate ET_AR and stimulate oxidative stress. Studies have shown an increase in BP and oxidative stress in ET_BR-deficient rats on HS diet, and ET_AR blockade prevents these effects, supporting a link between ET_BR deficiency and ET_AR-mediated increase in reactive oxygen species [87]. ET-1 stimulates superoxide production in the vasculature and may contribute to the increased oxidative stress and BP in salt-sensitive HTN, and the elevated BP during ET_BR blockade or infusion of ET-1 is attenuated by antioxidants. Also, the superoxide dismutase mimetic tempol attenuates the development of HTN in the initial days of rat treatment with an ET_BR antagonist, supporting that superoxide contributes to the increased BP during ET_BR deficiency and HS diet [87].

Modulators of Endothelin Receptors in HTN

Chronic treatment with the ET_AR/ET_BR antagonist bosentan or the ET_AR antagonist ABT-627 attenuates HTN and vascular remodeling in DOCA-salt rats, suggesting that ET_AR plays a major role in salt-sensitive HTN [88]. The net benefit of ET receptor antagonists in HTN could depend on their effectiveness to suppress the vasoconstrictive and growth promoting effects of ET. For instance, in hypertensive rats overexpressing ET-1, selective ET_AR antagonists slightly lower BP, but markedly attenuate vascular hypertrophy, particularly in resistance arteries. Similarly, treatment of SHR and DOCA-salt hypertensive rats with bosentan reduces BP, but abolishes the vascular hypertrophy and remodeling of resistance arteries beyond what could be explained by the BP lowering effect [77]. In healthy subjects, ET_AR antagonists alone or in combination with ET_BR antagonists do not modify basal vascular tone, while in essential hypertensive patients ET_AR blockade causes a slight vasodilation that is potentiated by ET_BR blockade. In line with a pathological role of ET-1 in essential HTN, bosentan lowers BP in patients with essential HTN. TAK-044 (ET_AR/ET_BR antagonist) and sitaxsentan (selective ET_AR antagonist) have shown some benefits in HTN, and the ET_AR antagonist darusentan has shown promising results in patients with resistant HTN [77]. However, sitaxsentan has recently been removed from the market by the manufacturer. Also, the more recent DORADO and DORADO-AC phase III large clinical trials evaluating darusentan for treatment of resistance HTN, failed to achieve the co-primary efficacy endpoint of changes in trough sitting systolic and diastolic BP from baseline to week 14 [89]. Although the study was 95% powered to detect an 8 mmHg improvement in systolic and diastolic BP, reductions in mean trough sitting systolic and diastolic BP were not statistically different between darusentan and placebo groups, and

therefore, the use of darusentan has been terminated for the treatment of resistance HTN [89].

One of the common side effects of mixed ET_AR/ET_BR antagonists or selective ET_AR-antagonists is hemodilution, fluid retention and edema [83] [90]. Blockade of renal ET_BR and probably ET_AR may reduce sodium excretion, and the resulting fluid retention may explain the failed clinical trials of ET receptor antagonists in the treatment of cardiac failure. The lack of efficacy of ET receptor antagonists against HTN in human trials suggest that the animal studies may not translate well in the clinical setting. However, larger clinical trials may be needed to determine any potential benefits of modulators of the ET system in HTN. ET receptor antagonists may not only decrease BP in HTN, but could also, on the long-term, prevent target organ damage and other cardiovascular complications associated with HTN such as PAH, CAD, restenosis after angioplasty, and atherosclerosis [77].

ET_BR in Pulmonary Circulation and Dysregulation in Pulmonary Hypertension

ET-1 plays an important role in the pulmonary circulation. In isolated rat intrapulmonary arteries ET-1 induced marked contraction, and bosentan, a dual ET_AR/ET_BR antagonist, and ambrisentan, an ET_AR antagonist with >4000-fold higher selectivity over ET_BR, relaxed ET-1 induced contractions by 30%, and 26%, respectively. Combination of the phosphodiesterase inhibitor tadalafil with bosentan relaxed ET-1-contracted arterial rings by 53%, and a combination of tadalafil with ambrisentan acted synergistically to relax arterial rings by 83%. Endothelium removal abolished the vasodilator response to tadalafil and its synergistic vasorelaxant effect with ambrisentan. Also, in the presence of the selective ET_BR antagonist BQ-788 the vasorelaxant effects of ambrisentan and tadalafil were additive but not synergistic. These data suggest that ambrisentan and tadalafil synergistically inhibit ET-1-induced constriction of rat intrapulmonary arteries and that endothelial ET_BR is necessary to enable a synergistic vasorelaxant effect of the drug combination [91]. Also, blockade of ET_BR induces vasoconstriction in perfused rat lung [92] and in rat and rabbit pulmonary artery [93], likely due to reduced vasodilator effects of endothelial ET_BR as well as inhibition of ET_BR-mediated ET-1 clearance and thereby enhancement of ET-1/ET_AR activity. On the other hand, in perfused rat lung, ET-1 induces pulmonary vasoconstriction that is inhibited by ET_AR/ET_BR blockade (BQ123+BQ788) more effectively than BQ123 alone, suggesting that both ET_AR and likely VSM ET_BR mediate ET-1 contraction [92]. Also, the mitogenic effects of ET-1 in pulmonary VSMCs appear to involve both ET_AR and ET_BR [94].

The ET-1 system appears to be upregulated during hypoxia. ET-1 levels are increased in the lungs of patients with pulmonary hypertension (PH) [95]. Also, the levels of preproET-1 and ET-1 peptide and ET_AR and ET_BR mRNA and protein are increased in the chronically hypoxic, hypertensive rat lung [10]. ET_BR is also important in the regulation of pulmonary vascular tone in response to hypoxia, and alterations in ET_BR activity may lead to increased pulmonary vasoconstriction, vascular remodeling and PAH. Chronic hypoxia augments ET_BR-mediated vasodilation in isolated perfused rat lungs [96]. Deficiency of pulmonary vascular ET_BR predisposes to the development of PH. The ET_BR antagonist BQ788 exacerbates hypoxia-induced pulmonary vasoconstriction in rats and causes PH and vascular remodeling in the ovine fetus [97]. Also, ET_BR-deficient rats develop PH and exaggerated pulmonary vasoconstriction when exposed to acute hypoxia or ET-1 stimulation. Transgenic *sl/sl* rats lack ET_BR mRNA expression in the pulmonary vasculature and develop PH, increased right ventricular hypertrophy and muscularization of small distal pulmonary arteries. Although steady-state mRNA levels of prepro-ET-1 are not elevated in transgenic *sl/sl* as compared to wild-type lungs, ECE-1 mRNA is higher, thus contributing to the increase in circulating ET-1 [98]. In ET_BR-deficient rats the pulmonary vessels show diminished endothelial NO synthase (eNOS) and NO production, supporting a role of NO in

ET_BR-mediated vasodilation in the pulmonary vasculature. Other studies in hypoxic pulmonary hypertensive rats have shown a decrease in PGI₂ synthase and PGI₂ production in ET_BR-deficient rat lung [98]. These findings suggest that ET_BR may provide a protective role and attenuate PH during chronic hypoxia. Also, in a rat model of monocrotaline-induced PH, an intravenous single bolus injection of ET-1 (1000 pmol/kg) caused a transient decrease in right ventricular systolic pressure (which is equal to the pulmonary arterial pressure) likely due to activation of endothelial ET_BR, and this effect was not observed in control rats. In rats pretreated with the ET_AR antagonist BMS-193884, ET-1 decreased right ventricular systolic pressure to a greater extent in monocrotaline-induced PH rats than in controls, likely due to greater activation of vasodilator ET_BR in ECs of the pulmonary circulation. Pretreatment with the dual ET_AR/ET_BR antagonist SB-209670, abolished ET-induced decrease in right ventricular systolic pressure in both groups. It was concluded that ET_AR antagonist may be more beneficial in the monocrotaline model of PH as it spares the enhanced activation of vasodilator ET_BR in ECs [99]. This is supported by the observation that ET_BR deficiency accelerates the progression of PH and neointimal lesions in monocrotaline-treated rats [98]. Also, pulmonary arterial ET-1 levels are 8-fold higher in the lungs of ET_BR-deficient than wild-type rats [98]. Interestingly, in ET_BR-deficient rats, ET_AR-induced vascular contraction is reduced despite elevated plasma ET-1 and increased ET_AR expression, suggesting uncoupling of ET_AR expression from functional activity [100]. In dihydromonocrotaline-treated dogs, the ET_AR antagonist FR139317 reduces both systemic and pulmonary vascular resistance, while the ET_BR antagonist RES-7011 increases pulmonary arterial pressure [101]. Other studies have shown the emergence of ET_BR-mediated vasoconstriction and ET_BRs in pulmonary VSMCs of fetal lambs with experimental congenital heart disease and increased pulmonary blood flow [102].

Although ET receptor antagonists have not been very effective in systemic vascular disease, they appear to be more efficacious in PAH, and the differences in their efficacy have been partly related to their ability to alter VSMC migration [103]. It was found that ET-1 potently induced migration of pulmonary VSMCs that was inhibited by selective ET_AR antagonist or combined ET_AR/ET_BR antagonist, but not by a selective ET_BR antagonist, whereas ET-1 had minimal effect on migration of aortic VSMCs. The ET-1 induced pulmonary VSMC migration appeared to involve activation and phosphorylation of ERK_{1/2} MAPK pathway and was less apparent in aortic VSMCs [103].

In addition to modulators of the NO and PGI₂ pathway, ET receptor antagonists have been shown in randomized controlled clinical trials to confer improvements in functional status, pulmonary hemodynamics, and even slow the progression of PAH [104]. The ET_AR antagonist ambrisentan at a dose of 5 to 10 mg daily and the combined ET_AR/ET_BR antagonist bosentan at a dose of 125 mg b.i.d have been approved for treatment of PAH in patients with a World Health Organization (WHO) functional class II to IV symptoms to improve exercise capacity and delay clinical worsening [105]. Combined ET_AR/ET_BR antagonists and selective ET_AR antagonists may also improve the hemodynamics in PAH patients with chronic heart failure (CHF) [106]. The ET antagonist lowering effects of pulmonary arterial pressure may be modest in the short-term, but could have significant long-term benefits. For example, in PAH, ambrisentan causes relatively small improvement in the short-term, but such improvement could be meaningful as one-year survival is improved [106]. Some small clinical trials have shown beneficial effects of bosentan (500 ng -1g, b.i.d for 2 weeks on the hemodynamics, CHF and PAH [107]. However, the REACH-1 and ENABLE studies demonstrated that initiation of bosentan therapy 125 to 500 mg b.i.d is associated with increased risk of worsening CHF [108]. Also, the RITZ-1 to -5 and VERITAS studies tested the effects of tezosentan 25 to 100 mg/hr for 24 hr in improving acute heart failure as measured by changes in dyspnea, oxygen saturation and cardiac index, but no benefits were observed [109].

Although both selective ET_AR and dual ET_AR/ET_BR antagonists could be beneficial in PAH, it is difficult to ascertain whether these agents provide equivalent ET_AR antagonism, or whether ET_BR blockade provides additional benefit. Because VSM ET_BR may contribute to the detrimental effects of ET-1 in PAH, complete blockade of the vasoconstrictive, proliferative and profibrotic effects of ET-1 by blocking both ET_AR and ET_BR may confer more benefits. However, these benefits may be offset by the loss of the protective role of endothelial ET_BR. Although most dual ET_AR/ET_BR antagonists show greater affinity toward ET_AR than ET_BR [110], they may block endothelial ET_BR mediated vasodilator effects. Also, while selective ET_AR antagonists are 100-fold more selective for ET_AR than ET_BR, higher doses of ET_AR antagonist may block ET_BR [110], and thereby suppress endothelial ET_BR-mediated vasodilation and impair ET_BR induced ET-1 clearance. Larger clinical trials are needed to determine the short-term and long term benefits of ET receptor antagonists in PAH and CHF.

ET_BR in Coronary Circulation and Dysregulation in Coronary Artery Disease

ET-1 is an important regulator of the coronary circulation. In the rat perfused heart, administration of ET-1 at low doses causes a reduction in coronary perfusion pressure, whereas at higher doses ET-1 causes a rise in coronary perfusion pressure [111]. Similarly, in anesthetized dogs, intracoronary bolus injection of lower doses (0.1 or 0.3 µg) of S6c caused a transient decrease in coronary resistance, likely via activation of endothelial ET_BR, whereas higher doses (1 and 3 µg) caused a decrease in coronary diameter and blood flow likely via VSM ET_BR [112]. *In vitro* experiments suggest that ET_AR rather than ET_BR plays a more prominent role in coronary vasoconstriction [113] [114]. In human coronary artery, ET-1 induced vasoconstriction is inhibited by the ET_AR antagonist BQ123 but not the ET_BR antagonist BQ788, supporting that ET-1 induced vasoconstriction is mainly through activation of ET_AR [114]. Also, immunoblots and immunofluorescence staining revealed that ET_AR expression is more predominant in the human coronary microvasculature, and ET_BR seems to be less abundant, supporting a greater role for ET_AR activation in coronary vasoconstrictor [114]. Also, in coronary arteries isolated from young and healthy pigs, ET-1 causes greater constriction than the ET_BR agonist IRL-1620. BQ788, endothelium removal or L-NAME potentiates the constrictor response to ET-1 and IRL-1620, suggesting ET_BR-mediated release of NO [115].

However, in clinical and experimental ischemic heart disease, VSM ET_BR appears to be upregulated in the coronary arteries and may play a role in promoting vasoconstriction [113]. VSM ET_BR is upregulated in coronary arteries after organ culture, a technique used to mimic the conditions of the coronary arteries in ischemic heart disease [116]. In rat coronary artery, S6c normally induces a small vasoconstrictor response, but after organ culture for 24 hr, S6c induces strong vasoconstriction, indicating the presence of contractile ET_BR [117]. Similar response to S6c after organ culture has been observed in porcine coronary artery [47]. The strong contractile effect of S6c in blood vessels in organ culture may be related to upregulation of contractile ET_BR in VSMCs and/or loss of functional endothelium and its vasodilatory ET_BR. ET_BR mRNA and protein levels are enhanced in rat coronary arteries following organ culture [117], and upregulation of contractile ET_BR is inhibited by actinomycin D and cycloheximide, supporting a role of transcription and translation of ET_BR, respectively. Coronary arteries in organ culture upregulate vasoconstrictor ET_BR via transcriptional mechanisms and MEK-ERK_{1/2} signaling [116]. Minimally modified low density lipoprotein (LDL), a risk factor of CAD, also upregulates ET_BR in rat coronary VSMCs, mainly via activation of the ERK_{1/2} MAPK and the downstream transcriptional factor NF-κB [118].

ET-1 is upregulated in patients with CAD. Intravenous administration of bosentan in patients with stable CAD decreased BP and increased coronary diameter particularly in

segments with mild angiographic changes, and the coronary vasodilator effects of bosentan were inversely related to plasma LDL cholesterol levels [119]. Also, patients with ischemic heart disease have elevated plasma levels of ET-1 and AngII and upregulation of ET_BR and AT₁R in subcutaneous arteries when compared with healthy controls [120], suggesting that VSM ET_BR may play a role in the pathology of CAD and could provide a target for pharmacological intervention. The observations that ET-1 induces coronary vasoconstriction and that VSM ET_BR constrictor response is enhanced in the coronary vasculature during clinical and experimental ischemic heart disease suggest that mixed ET_AR/ET_BR antagonists could be more beneficial than selective ET_AR antagonists.

ET_BR in Cerebral Circulation and Dysregulation in Cerebral Ischemia

ET_BR plays an important role in the cerebral circulation. In intact rat cerebral artery treated with the ET_AR antagonist FR139317, intraluminal administration of S6c causes concentration-dependent vasodilation, suggesting a role of endothelial ET_BR in cerebral vasodilation [121]. Also, in freshly isolated rat middle cerebral arteries, ET-1 elicits a strong contraction that is almost totally dependent on ET_AR, while S6c shows no or weak contractile effect [122]. However, during cerebral ischemia there appears to be a switch from vasodilator endothelial ET_BR to the vasoconstrictor VSM ET_BR. For instance, ET_BR is upregulated in human cerebral arteries in organ culture particularly under conditions that mimic those observed in cerebral ischemia [123]. Also, in rat cerebral arteries in organ culture for 24 to 48 hr, S6c causes upregulation of VSM ET_BR and produces a strong contraction [122]. Similarly, in rat cerebral arteries cultured for 24 hr treatment with tumor necrosis factor- α or epidermal growth factor potentiates S6c-induced contraction, suggesting that cytokines and growth factors can enhance the expression of VSM ET_BR in cerebral arteries and perhaps during cerebral ischemia [124]. Interestingly, minimally modified LDL, a risk factor for cerebral vascular disease, also upregulates VSM ET_BR in rat basilar artery [125]. The upregulation of ET_BR in cerebral VSMCs in organ culture may involve activation of PKC, CAMK-II, B-Raf/MEK/ERK_{1/2} MAPK and NF- κ B [125].

Endothelial ET_BR may also confer neuroprotective effects in cerebral ischemia. In a rat model of focal cerebral ischemia induced by middle cerebral artery occlusion, treatment with IRL-1620 improves neurological and motor function and reduces oxidative stress and infarct volume, suggesting that endothelial ET_BR activation may be a therapeutic target for the treatment of focal cerebral ischemia and stroke [126]. Cerebral vasospasm develops in 30–70% of patients suffering from subarachnoid hemorrhage, leading to delayed cerebral ischemia, permanent neurological dysfunction and death. Cerebral vasospasm is associated with elevated ET-1 levels in the cerebrospinal fluid, implicating ET-1 as one of the factors in the pathogenesis of cerebral ischemia [127]. Studies have shown that the selective ET_AR antagonist clazosentan reduces vasospasm in a Phase 2a blinded, placebo-controlled trial in patients with subarachnoid hemorrhage [127]. However, it remains uncertain as to whether clazosentan improves morbidity and mortality as these endpoints were not measured. Also, given that the contractile VSM ET_BR could be upregulated during cerebral ischemia, the potential effects of dual ET_AR/ET_BR antagonists in the setting of subarachnoid hemorrhage may need to be examined.

Dysregulation of ET_BR in Vascular Inflammation and Atherosclerosis

ET_BR may play a role in conditions associated with vascular inflammation and atherosclerosis. Competitive binding studies *in vitro* revealed that ET_BR are upregulated in coronary arteries from patients with atherosclerosis [128]. Also, accumulation of foamy macrophages and T-lymphocytes in human atherosclerotic lesions may cause a switch from ET_AR to ET_BR in VSMCs, suggesting that ET_BR may play a more important role during the progression of atherosclerosis [129]. Studies have compared the effects of infusing ET-1 and

S6c into the brachial artery while forearm blood flow (FBF) is measured in patients with atherosclerosis and healthy control subjects. ET-1 induced a vasoconstriction response that was similar in the two groups. In comparison, S6c evoked an initial vasodilation and increase in FBF that did not differ between the two groups, followed by vasoconstriction and reduction in FBF that were greater in the atherosclerotic patients than in controls, and correlated with LDL cholesterol levels, suggesting upregulation of VSM ET_BR in atherosclerosis [130]. Intra-arterial infusion of the ET_BR antagonist BQ788 evoked an increase in FBF in patients with atherosclerosis, but a 9% reduction in control subjects. BQ123+BQ788 evoked an increase in FBF in the patients but not control subjects. The increase in FBF evoked by selective ET_AR blockade was less than that evoked by combined ET_AR/ET_BR blockade in atherosclerotic patients, suggesting an enhanced ET-1 mediated vascular tone, at least in part due to increased ET_BR-mediated vasoconstriction [131]. *In vitro* and *in vivo* studies in mice have also demonstrated a shift from ET_AR to ET_BR in arteriosclerotic vessels [132].

Studies have examined coronary tone and endothelial function before and after infusion of ET antagonists into unobstructed coronary arteries of patients with coronary atherosclerosis. BQ788 did not affect epicardial diameter but constricted the microcirculation, increased coronary sinus ET-1, and reduced NO levels likely due to reduced ET_BR-mediated ET-1 clearance and NO availability. BQ123+BQ788 dilated epicardial and resistance arteries and improved endothelial dysfunction of epicardial arteries. It was concluded that ET-1 via ET_AR contributes to basal constrictor tone and endothelial dysfunction, while ET_BR mediates vasodilation in human coronary arteries with atherosclerosis [133]. ET_AR blockade reduces atheroma plaque formation in apolipoprotein E-deficient mice [4]. Also, ET_BR may mediate favorable inhibition of vascular remodeling after injury as supported by exaggerated injury in ET_BR KO mice [134]. These observations suggest that selective ET_AR antagonists may have greater therapeutic potential than nonselective agents in conditions associated with vascular inflammation and atherosclerosis [133].

ET_BR, Sex Differences, Pregnancy, and Early-Life programming

ET_BR may be modulated differently between sexes. Studies have shown sex difference in the development of salt-sensitive superoxide-dependent HTN in ET_BR-deficient rats, with females having greater increases in BP and plasma ET-1 levels in response to HS diet than males [135]. Also, in the rat model of carotid artery balloon injury and neointima formation, the neointima/media ratio is lower in females than males, and the sex difference is attenuated by ovariectomy and restored by treatment with 17 β -estradiol. In ET_BR-deficient rats, the neointima/media ratio increased to the same level in both male and female rats, and this increase was not affected by ovariectomy or 17 β -estradiol treatment. Treatment with A-192621, a selective ET_BR antagonist, abolished the sex difference in balloon injury-induced neointima formation. These findings indicate that the sex differences in balloon injury-induced neointimal formation is abolished by genetic ET_BR deficiency or its pharmacological blockade [136]. Thus sex steroids may modulate the vascular ET_BR, and such effects may play a role in the sex differences in vascular function and the vascular changes associated with menopause.

ET_BR may also play an important role during pregnancy. The ET_AR antagonist A-127722 or FR-139317 lowers BP in both pregnant and nonpregnant rats, whereas the ET_BR antagonist A-192621 causes HTN in pregnant rats, supporting a role of ET_BR in modulating BP during pregnancy [137]. Also, relaxin may upregulate endothelial ET_BR during pregnancy [138]. Preeclampsia is a pregnancy-associated disorder characterized by HTN, proteinuria and endothelial dysfunction. Placental ischemia/hypoxia could lead to increased synthesis and release of ET-1 in preeclampsia [139]. Plasma ET-1 levels are 2- to 3-fold higher in preeclamptic than normal pregnant women, and are highest during the late stages of the

disease, suggesting a role of ET-1 in the progression into the malignant phase of preeclampsia rather than the initiation of the disease. The role of vascular ET_AR and ET_BR during pregnancy, and the potential effects of ET_AR/ET_BR antagonists in preeclampsia need to be further examined.

Early life stress may downregulate ET_BR expression and increase acute stress-mediated BP in adult rats. In male wild-type and ET_BR-deficient rats subjected to early life stress by maternal separation for 3 hr/day from day 2 to 14 of postnatal life, acute air jet stress caused elevation of BP, plasma corticosterone and plasma ET-1 levels in adult maternally separated rats compared with control non-handled littermates. Maternal separation and early life stress also caused downregulation of ET_ARs and ET_BRs in aortic tissue compared with control rats, which could lead to alterations in the ET pathway, and an exaggerated acute pressor response to stress in adulthood [140].

Conclusions and Future Directions

In addition to ET_AR, ET_BR is important for the control of vascular reactivity and BP. The unique anatomical location of endothelial ET_BR favors both the release of endothelium-derived vasodilators, which counteract the protracted vasoconstrictor effects of ET-1/ET_AR, and clearance of ET-1 from the circulation. ET_BRs are also present in VSM and share several intracellular signaling pathways with the ET_AR. The availability of selective ET_BR agonists and antagonists and the development of transgenic ET_BR-deficient animals have contributed to our understanding of the role of ET_BR in the control of vascular function, and the changes in its role in pathological conditions and CVD. Clinical evidence supports vascular benefits of ET antagonists in treatment of PAH, and potential benefits in atherosclerosis, CHF, and renal failure. However, there is still a debate as to the benefits of ET_AR antagonists alone vs. mixed ET_AR/ET_BR antagonists in CVD. Dual ET_AR/ET_BR antagonists such as bosentan, enrazaten and tezosentan may be beneficial in CHF and PAH. However, recent reports favor the use of selective ET_AR antagonists over dual ET_AR/ET_BR antagonists [38]. Combined ET_AR antagonist with ET_BR agonist may need to be evaluated in conditions associated with decreased endothelial ET_BR expression/activity. Targeting ECE-1 may also provide a new approach to alter the production of ET-1 in CVD.

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List of abbreviations

AngII	angiotensin II
AT₁R	angiotensin type 1 receptor
BP	blood pressure
[Ca²⁺]_i	intracellular free Ca ²⁺ concentration
CAD	coronary artery disease
CAMK-II	Ca ²⁺ /calmodulindependent protein kinase II
CHF	chronic heart failure
DAG	diacylglycerol
DOCA	deoxycorticosterone acetate

ECE	endothelin converting enzyme
ECs	endothelial cells
EDHF	endothelium-derived hyperpolarizing factor
EDNRB gene	endothelin receptor type B gene
ET-1	endothelin-1
EDHF	endothelium-derived hyperpolarizing factor
ET_AR	endothelin receptor type A
ET_BR	endothelin receptor type B
FBF	forearm blood flow
GPCR	G-protein coupled receptor
GRK	G-protein coupled receptor kinase
HS	high salt
HTN	hypertension
IP₃	inositol 1,4,5-trisphosphate
KO	knockout
L-NAME	N _ω -nitro-L-arginine methyl ester
LDL	low-density lipoprotein
MAPK	mitogen-activated protein kinase
MLC	myosin light chain
NO	nitric oxide
PGI₂	prostacyclin
PAH	pulmonary arterial hypertension
PKC	protein kinase C
PLC-β	phospholipase C-β
S6c	sarafotoxin c
SHR	spontaneously hypertensive rats
TMD	transmembrane domains
VSM	vascular smooth muscle

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Fig. 1.

Human ET_BR structure. ET_BR is composed of a long N-terminus, seven helical transmembrane domains (TMD), 3 extracellular loops, 3 intracellular loops, and an intracellular C-terminal tail. The receptor has conserved GPCR DRY sequence and Cys402/403/405. Mutations in some of these sites could affect receptor binding/signaling. Also, palmitoylation, phosphorylation and glycosylation of ET_BR could affect intracellular signaling.

Fig. 2.

ET_BR-mediated signaling pathways in the vascular system. In endothelial cells (ECs), preproET-1 is cleaved into big ET-1 which is cleaved by endothelin-converting enzyme (ECE) into active ET-1. ET-1 is released from ECs, and acts in a paracrine fashion to stimulate ET_BR and ET-1 clearance. ET_BR also activates phospholipase C- β (PLC β) and increases the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ induces Ca²⁺ release from the endoplasmic reticulum (ER). The increased [Ca²⁺]_i activates endothelial nitric oxide synthase (eNOS) and increases nitric oxide (NO) production. NO diffuses into vascular smooth muscle (VSM), where it stimulates guanylate cyclase (GC) and increases cGMP. cGMP causes vascular relaxation by decreasing Ca²⁺ entry through Ca²⁺ channels, stimulating Ca²⁺ removal via plasmalemmal (PMCA) and sarcoplasmic reticulum Ca²⁺-ATPase (SERCA), and decreasing the actin-myosin myofilament force sensitivity to Ca²⁺. Endothelial ET_BR is also coupled to stimulation of cyclooxygenases (COX-2) and increased prostacyclin (PGI₂). PGI₂ activates adenylate cyclase (AC) and increases cAMP, which causes VSM relaxation by mechanisms similar to those of cGMP. Activation of endothelial ET_BR also increases the release of endothelium-derived hyperpolarizing factor (EDHF), which activates K⁺ channels and causes VSM hyperpolarization and relaxation. In VSM, the interaction of ET-1 with ET_BR activates PLC β and increases IP₃ and DAG. IP₃ stimulates Ca²⁺ release from the sarcoplasmic reticulum (SR). ET-1 also stimulates Ca²⁺ entry through Ca²⁺ channels. Ca²⁺ binds calmodulin to form a complex, which causes activation of myosin light chain (MLC) kinase (MLCK), MLC phosphorylation (MLC-P), actin-myosin interaction and VSM contraction. DAG activates PKC, which phosphorylates the actin-binding protein calponin (CAP) or initiates a protein kinase cascade involving Raf, MAPK kinase (MEK) and MAPK (ERK_{1/2}), leading to phosphorylation of caldesmon (CAD) and

thereby increases the myofilament force sensitivity to Ca^{2+} . $\text{ET}_\text{B}\text{R}$ could also activate Rho-kinase, which inhibits MLC phosphatase and increases MLC phosphorylation. $\text{ET}_\text{B}\text{R}$ -mediated activation of $\text{ERK}_{1/2}$ MAPK could also induce gene transcription and VSM growth and proliferation. Dashed arrows indicate inhibition.

Fig. 3.

ET receptor agonists and antagonists. ET peptides include ET-1, ET-2 and ET-3 which share structure homology with the snake venom sarafotoxin b (S6b) and c (S6c) (A). Other ET_BR agonists include the synthetic linear analogs [Ala¹,3,11,15]ET-1, BQ3020 and IRL-1620 (B). ET_BR antagonists include BQ788, IRL-1038, IRL-2500 and non-peptide A-192621 (C). Commonly used experimental ET_AR antagonists include BQ123 (D). Clinically available or under trials drugs include the mixed ET_AR/ET_BR antagonist bosentan, and the selective ET_AR antagonists ambrisentan, darusentan and sitaxsentan (E).

Table 1

Structure, Distribution, Signaling Pathways, Functions, and Representative Agonists and Antagonists in ET_BR Subtypes as Compared to ET_AR

	Endothelial ET _B R	VSM ET _B R	ET _A R
Structure	GPCR, 7 TMD, 442 aa ~50kDa	GPCR, 7 TMD, 442 aa ~50kDa	GPCR, 7 TMD, 427 aa ~59kDa
Order of Potency	ET-1=ET-2=ET-3	ET-1=ET-2=ET-3	ET-1=ET-2>>ET-3
Selective Agonists	[Ala1,3,11,15]ET-1 BQ3020 IRL-1620 S6c	[Ala1,3,11,15]ET-1 BQ3020 IRL-1620 S6c	N/A
Selective Antagonists	A-192621 BQ788 IRL-1038 IRL-2500 RES-7011 Ro-468443	A-192621 BQ788 IRL-1038 IRL-2500 RES-7011 Ro-468443	ABT-627 BQ123 BQ610
Vascular Distribution			
Coronary Artery	+	+	+
Subcutaneous Arteries	+	+	+
Pulmonary Artery	+	+	+
Mammary Artery	+	+	+
Veins	+	+	+
Glomerular Capillaries	+	+	+
Cellular Distribution	Endothelium	VSM	VSM
Sub-Cellular Distribution	Cytosol, plasmalemma, nucleus, nucleoplasm	Cytosol, plasmalemma, nucleus, nucleoplasm	Cytosol, plasmalemma, nucleus
G Protein	G Protein	G _q , G ₁₁ , G _{12/13} , G _i , G _o	G _q , G ₁₁ , G _{12/13} , G _i , G _o
Signaling Pathways	<ul style="list-style-type: none"> Increased [Ca²⁺]_i NO PGI₂ EDHF 	<ul style="list-style-type: none"> PLCβ, PLD Increased [Ca²⁺]_i PKC MAPK 	<ul style="list-style-type: none"> PLCβ, PLD Increased [Ca²⁺]_i PKC MAPK
Effect on VSM	Relaxation	Contraction	Contraction and growth
Effects on Blood pressure and Vascular function	<ul style="list-style-type: none"> ↓ BP ET-1 clearance ↑ Vasodilation via NO, PGI₂, EDHF, K⁺ channels ↑ Renal medullary blood flow via NO and PGI₂ production ↓ Renin release ↑ Na⁺ and water excretion Counteracts ET_AR-mediated vascular 	<ul style="list-style-type: none"> ↑ BP ↑ Sensitivity of contractile actions to ET-1 ↑ vascular resistance ↑ vasoconstriction 	<ul style="list-style-type: none"> ↑ BP ↑ Sensitivity of contractile actions to ET-1 ↑ Vasoconstriction ↑ afferent and efferent arteriolar resistance Vascular remodeling ↓ Renal Blood flow and glomerular filtration rate

	Endothelial ET _B R	VSM ET _B R	ET _A R
	and renal tubular actions		

Table 2**ET-1/ET_BR-Related Vascular Phenotype Associated with Experimental HTN**

Animal	Characteristics	Reference
Normal Sprague-Dawley Rats	<ul style="list-style-type: none"> ET-1 causes a transient depressor effect via endothelial ET_BR followed by a sustained pressor response through activation of ET_AR and possibly ET_BR in VSM. ↑ BP during infusion of ET_BR antagonist A192621 particularly during HS diet 	[3] [57] [80]
DOCA-salt sensitive rats	<ul style="list-style-type: none"> ↑ Circulating and tissue (kidney and aorta) ET-1 levels ↑ Urinary Excretion of ET ↑ Density of ET_BR in kidneys ↓ Plasma renin Chronic ET_AR Blockade ↓ HTN and renal injury 	[28] [88]
Dahl salt-sensitive rats	<ul style="list-style-type: none"> ↓ET_BR expression in renovascular endothelium BQ3020 ↓ renal perfusion pressure and NO production 	[141]
Transgenic rescued ET _B R-deficient rats	<ul style="list-style-type: none"> ↑ Circulating and tissue (kidney and aorta) ET-1 levels ↑ Urinary ET ↓ Plasma renin Severe HTN during HS diet, renal dysfunction Chronic ET_AR blockade ↓ HTN and renal injury Normal BP restored with epithelial sodium channel blocker amiloride. ↑ Aortic superoxide production and plasma 8-isoprostane ↑ Neointimal lesions after balloon injury 	[14] [87] [55] [98]
Transgenic rescued ET _B R-deficient mice	<ul style="list-style-type: none"> ↑ BP ↓ ET-1 clearance ↓ Endothelium-dependent relaxation in mesenteric arteries ET_AR mediate vasoconstriction in afferent arterioles ET_BR has no vasoconstrictor or vasodilator action in afferent arterioles ↓ Endothelial ET_BR mediated vasodilation in efferent arterioles ↓ VSM ET_BR-mediated vasoconstriction but intact ET_AR mediated vasoconstriction in efferent arterioles 	[38] [54] [82]

Table 3

ET_BR-Mediated Systemic and *in vivo* Actions, and *in vitro* Effects on Representative Vascular Preparations from Different Species

Species	Tissue/Preparation	Receptor/Cell	Effect	Ref
Mouse	Systemic	ET _B R / EC ET _B R / VSM	Depressor effect and ET-1 clearance Sustained pressor effect on BP	[38] [54]
	Aorta	ET _B R	Predominant vasodilator, but also small vasoconstrictor	[56]
	Renal afferent arterioles Renal efferent arterioles	Absent ET _B R / EC ET _B R / VSM	No vasodilation or constriction Vasodilation Vasoconstriction	[38] [82]
Rat	Systemic	ET _B R / EC ET _B R / VSM ET _B R ET _B R / VSM ET _B R / VSM	Transient depressor effect Sustained pressor effect ET-1 Clearance ↑ Total vascular resistance, ↓ cardiac output Mesenteric and renal vasoconstriction	[57] [58] [59] [60] [61]
	Aorta	ET _B R / EC	Increased endothelium-dependent relaxation via NO	[142]
	Mesenteric arteries	ET _B R ET _B R / VSM	Fresh microvessels: variable vasoconstriction Organ culture: vasoconstriction	[3] [4] [38]
	Cerebral and basilar microvessels	ET _B R / VSM	Fresh microvessels: little and variable vasoconstriction Organ culture: vasoconstriction	[45] [125]
	Carotid	ET _B R / EC	Vasodilation	[39]
	Perfused lung and pulmonary artery	ET _B R / VSM	Constrictor response	[92]
	Mesenteric vein	ET _B R / VSM	Constriction	[75]
	Vena cava	ET _B R / EC ET _B R / VSM	Relaxation Contraction	[72] [76]
Hamster	Systemic	ET _B R / EC	Depressor effect on BP	[81]
Rabbit	Pulmonary artery	ET _B R / VSM	Contraction	[93]
	Saphenous vein	ET _B R / VSM	Contraction	[74]
Pig	Coronary arteries	ET _B R / VSM	Fresh microvessels: little and variable vasoconstriction Organ culture: vasoconstriction	[47]
Dog	Coronary artery (<i>in vivo</i>)	ET _B R / EC ET _B R / VSM	Transient decrease in resistance Sustained decrease in blood flow and diameter	[112]
Human	Forearm resistance arteries (<i>in vivo</i>)	ET _B R / EC ET _B R / VSM	Vasodilation Vasoconstriction	[69] [70]
	Dorsal hand veins (<i>in vivo</i>)	ET _B R / VSM	Vasoconstriction	[70]
	Coronary artery (<i>in vivo</i>)	ET _B R / EC	Vasodilation	[133]
	Coronary artery	ET _B R / VSM	Little vasoconstrictor	[16]
	Cerebral artery	ET _B R / VSM	Organ culture: vasoconstriction	[123]
	Internal mammary artery	ET _B R / VSM	Vasoconstriction	[16] [71]
	Saphenous vein	ET _B R / VSM	Contraction	[73]