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ENDOTHELIN SYSTEM: The Double-Edged Sword in Health and Disease

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■ **Abstract** The endothelin system consists of two G-protein-coupled receptors, three peptide ligands, and two activating peptidases. Its pharmacological complexity is reflected by the diverse expression pattern of endothelin system components, which have a variety of physiological and pathophysiological roles. In the vessels, the endothelin system has a basal vasoconstricting role and participates in the development of diseases such as hypertension, atherosclerosis, and vasospasm after subarachnoid hemorrhage. In the heart, the endothelin system affects inotropy and chronotropy, and it mediates cardiac hypertrophy and remodeling in congestive heart failure. In the lungs, the endothelin system regulates the tone of airways and blood vessels, and it is involved in the development of pulmonary hypertension. In the kidney, it controls water and sodium excretion and acid-base balance, and it participates in acute and chronic renal failure. In the brain, the endothelin system modulates cardiorespiratory centers and the release of hormones. More advanced functional analysis of the endothelin system awaits not only additional pharmacological studies using highly specific endothelin antagonists but also the generation of genetically altered rodent models with conditional loss-of-function and gain-of-function manipulations.

INTRODUCTION

In the mid-1980s, an increasing awareness of the role of endothelial cells as active components of the vascular system led to the description of an endothelial cell-derived constricting factor (1, 2). In 1988, a 21-amino-acid vasoconstricting factor termed endothelin was isolated from cultured porcine aortic endothelial cells (3). This small peptide has an array of physiological functions, with roles in basal vascular tone, sodium balance, neural crest cell development, and neurotransmission. This review outlines the physiology and pathophysiology of endothelins in mammalian vessels, and heart, lung, kidney, and brain tissues. We also hint at future

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research directions, particularly those utilizing new genetically modified animal models.

Endothelin-1 (ET-1) is a 21-amino-acid peptide with a hydrophobic C terminus and two cysteine bridges at the N terminus. Within 1 year of its discovery, two structurally related peptides differing by two and six amino acids were identified and termed endothelin-2 (ET-2) and endothelin-3 (ET-3), respectively (4; Figure 1). The endothelin precursors are processed by two proteases to create the mature active forms. The ~200-residue preproendothelins are cleaved at dibasic sites by furin-like endopeptidase to form biologically inactive intermediates—37- to 41-amino-acid peptides termed big endothelins (big ETs). Next big ETs are

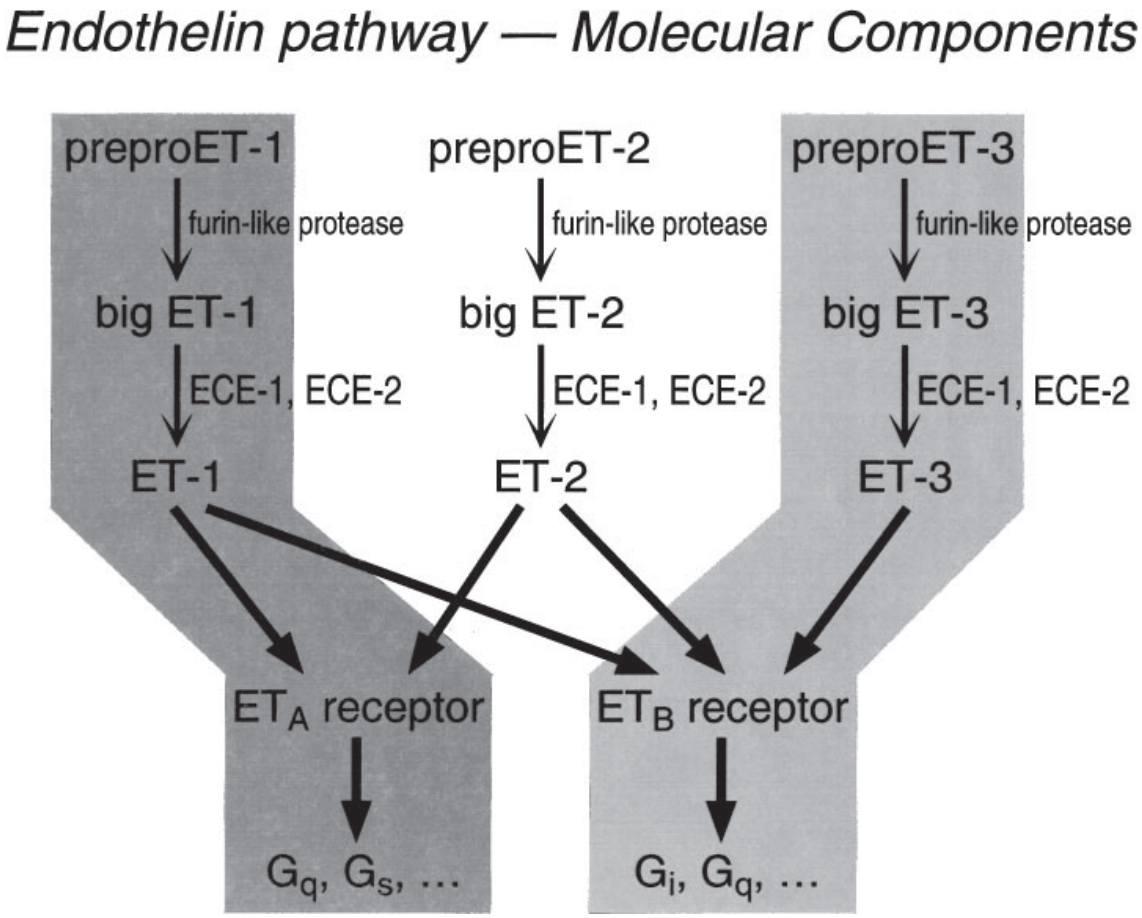

Figure 1 The endothelin pathway. Preproendothelins are processed by furin-like proteases to form big-ET intermediates that are further cleaved to three physiologically active endothelin peptides by endothelin-converting enzymes. The peptides interact with a pharmacologically selective ET_A receptor and nonselective ET_B receptor. These receptors couple to various G proteins to produce an array of different physiological responses. The left shaded column highlights the importance of ET-1, ET_A, and ECE-1 in the development of cephalic and cardiac neural crest-derived craniofacial and cardiac outflow structures, while the right shaded column tracks the importance of ET-3, ET_B, and ECE-1 in the development of neural crest-derived epidermal melanocytes and enteric neurons.

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cleaved at the Trp-Val of ET-1 and ET-2 or at the Trp-Ile of ET-3 to form the 21-amino-acid final products (4). This last processing step is carried out by a family of membrane-bound zinc metalloproteases from the neprilysin superfamily, termed endothelin-converting enzymes (ECEs). To date, two family members involved in big-ET processing have been identified. ECE-1 is found in a variety of cells including endothelial cells, and it has a sharp activity peak at neutral pH. It processes big ETs both intracellularly and on the cell surface (5). ECE-2 is located in diverse cell types including neurons, and it has a sharp activity peak at pH 5.8. The acidic activity makes ECE-2 a likely intracellular-processing enzyme (6). Both ECE-1 and ECE-2 show preference for big ET-1 over either big ET-2 or big ET-3 in vitro. Besides these two proteases, another unidentified enzyme(s) can carry out the final processing step, since mice lacking both ECE-1 and ECE-2 still contain significant levels of mature endothelin peptides (7). The clearance of endothelins is just as complex as their synthesis. In renal tissues, neutral endopeptidase (EC 3.4.24.11) controls turnover of ET-1, because the inhibitors of this endopeptidase increase urinary endothelin levels severalfold (8). The endothelin-B (ET_B) receptor also likely functions as a "clearance receptor," because the ET_B-selective antagonist BQ-788 significantly inhibits accumulation of intravenously administered, radiolabeled ET-1 in lungs and kidneys, thereby slowing its clearance from the circulatory system, whereas the endothelin-A (ET_A) receptor-selective antagonist BQ-123 has no such effect (Table 1). This ET_B-mediated clearance mechanism is particularly important in the lung, where 80% of ET-1 passing through this organ is retained (9).

In mammals, two endothelin (ET_A and ET_B) receptors have been identified (10, 11). Both contain seven transmembrane domains of 22–26 hydrophobic amino acids in their ~400-amino-acid sequences. Each receptor activates an overlapping set of G proteins, leading to diverse responses such as activation of phospholipase C-beta, increase in intracellular calcium, and induction of immediate early genes (12). The ET_A has subnanomolar affinities for ET-1 and ET-2 and $\geq$2-orders-of-magnitude-lower affinity for ET-3 (10). ET_B has equal subnanomolar affinities for all endothelin peptides (11). Based on its in vivo pharmacology, ET_B has been further classified into two types, ET_B1 and ET_B2, although the molecular basis for the existence of these subtypes is still lacking (13).

TABLE 1 Summary of endothelin antagonists

ETA antagonist	ETB antagonist	ETA/ETB antagonist
BQ-123	RES-701-1	TAK-044
BMS-182874	BQ-788	Bosentan
LU135252		
EMD 94246		
FR139317		
PD156707		

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Underlying the complex physiology of the endothelin system is the diverse pattern of expression of its components. ET-1 is made by endothelial cells, airway epithelial cells, macrophages, fibroblasts, cardiomyocytes, various brain neurons, and other cells (3, 14–22). In vivo, the predominant source of ET-1 is endothelial cells (23). ET-2 is expressed by intestinal epithelial cells, and ET-3 by brain neurons, renal tubular epithelial cells, and intestinal epithelial cells (24, 25). ET_A is found on vessel and airway smooth muscle cells, cardiomyocytes, liver stellate cells and hepatocytes, brain neurons, osteoblasts, melanocytes and keratinocytes, adipocytes, and various cells in the reproductive tract. ET_B exists on vessel endothelial cells and smooth muscle cells, liver hepatocytes and Ito cells, renal-collecting-duct epithelial cells, airway smooth muscle cells, osteoblasts, neurons of the central and peripheral nervous system, and various cells of the reproductive tract (26) (Table 2). In many tissues, therefore, the same cells express both receptors.

Regulation of peptidic mediators can occur at the levels of synthesis, storage, and release. Because many endothelin-producing cell types do not have storage vesicles or regulated secretory pathways, regulation of endothelin level takes place at synthesis, particularly transcription. In a variety of cells, *ET-1* mRNA is upregulated by transforming growth factor-beta, tumor necrosis factor-alpha, interleukins, insulin, norepinephrine, angiotensin II, and thrombin (3, 27, 28). In endothelial cells, *ET-1* mRNA is initially increased and then decreased by shear stress and stretch (29). The level of mRNA for *ET-1* is upregulated by hypocapnia and downregulated by hypoxia (30). In endothelial cells, *ET-1* mRNA is also decreased in response to nitric oxide, prostacyclin, and atrial natriuretic factor (31–33). A number of factors affect the expression of endothelin receptors. In smooth muscle cells, ET_A is upregulated by insulin and nitric oxide (34, 35). In endothelial cells, ET_B is upregulated by tumor necrosis factor-alpha and basic fibroblast growth factor (36).

TABLE 2 Expression pattern of endothelin system components in normal physiology

	ET-1	ETA	ETB
Endothelial cells	+		+
Smooth muscle cells		+	+
Cardiomyocytes	+	+	+
Hepatocytes	+	+	+
Renal collecting-duct cells	+		+
Neurons	+	+	+
Osteoblasts		+	+
Keratinocytes	+	+	+
Adipocytes		+	+

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As noted, complex interactions of local signals regulate the endothelin system at the levels of both the peptide and the receptor.

The concentration of ET-1 in plasma in many species is ~1 pM, 2 orders of magnitude below the pharmacological threshold. The circulating concentrations of big ETs are also ~1 pM, and plasma ET-2 and ET-3 are found at even lower concentrations (37). Therefore, under normal physiological conditions, endothelins are not circulating hormones; rather they act as autocrine and paracrine factors at multiple sites in the body. These diverse roles create a particular challenge in studying their physiology, because administration of endothelin antagonists can affect multiple organs simultaneously. This fact can limit the value of pharmacological experiments and create the necessity for animal models with modified expression of endothelin components in selected cell populations, such as conditional gene knockout and inducible transgenic animals.

PHYSIOLOGICAL ROLES

Endothelins in Development

Gene knockout experiments in mice have dramatically defined the importance of the endothelin system in development. ET-3, ET_B, and ECE-1 are necessary for the correct development of neural crest-derived epidermal melanocytes and enteric neurons (38–40). In contrast, ET-1, ET_A, and ECE-1 are necessary for the patterning and development of cephalic and cardiac neural crest-derived craniofacial and cardiac outflow structures (40–42).

ET-1 and ET_A knockout mice die at birth from mechanical asphyxia due to severe malformation of neural crest-derived facial and throat structures. Most ECE-1-deficient mice die much earlier in utero. ET-1-, ET_A-, or ECE-1-deficient animals have craniofacial malformations that include a cleft palate, poorly developed mandible, hypoplastic tongue, and abnormal fusion of the hyoid bone to the base of the skull (40–42). Abnormalities also exist in the middle ear structures. For correct patterning and proliferation of cephalic neural crest cells, ET_A expressed in neural crest cells and neural-derived mesenchymal cells must be activated by ET-1 secreted by epithelium of the pharyngeal arches around embryonic day 10 (43). The cardiac neural crest cells also contribute to the tunica media of the developing arch arteries and to the formation of the outflow septation. Mice lacking ET_A, ET-1, or ECE-1 display arterial defects that include the absence of the right subclavian artery, the interruption of the aorta, and the presence of a right-sided aortic arch. Ventricular septal defects are also commonly observed in these animals (40, 41, 44). Significantly more severe cardiac abnormalities are observed in mice lacking both ECE-1 and ECE-2 (7). Between embryonic days 9.5 and 11.5, ET-1 secreted by overlying endothelium must interact with ET_A on the overlying neural crest-derived mesenchymal cells for correct development of arterial structures (45).

ET-3 and ET_B gene knockout mice are viable at birth, but they fail to thrive and subsequently die at 3–6 weeks of age. ECE-1 knockout mice display the additive

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phenotypes of animals lacking ET-1/ET_A and ET-3/ET_B pathways. Therefore, ET-3-, ET_B-, or ECE-1-deficient animals lack epidermal melanocytes, resulting in normal retinal melanin pigment (black eyes) but white-spotted hair and skin color. In addition, the distal colon from the sigmoid colon to the rectum is narrowed and functionally obstructed by a lack of myenteric ganglia. The lack of enteric nervous system components leads to dilation of the proximal colon, intestinal dysfunction, and death (38–40). For the correct placement and differentiation of enteric nervous system precursors and epidermal melanoblasts, ET_B on these neural crest-derived cells must be activated by ET-3 secreted from mesenchymal tissue (46). This is further confirmed by in vitro experiments showing that ET-3-stimulated neural crest cells divide rapidly and then differentiate into melanocytes (47). Experiments in mice in which the *ET_B* locus has been placed under the control of tetracycline-dependant transactivators show that activation of the ET_B pathway between embryonic day 10 and 12.5 is necessary and sufficient for correct development of enteric neurons and melanocytes (48). Distal intestinal aganglinosis and the lack of epidermal melanocytes have also been identified in lethal-spotting (ls) and piebald-lethal (sl) mice that harbor naturally occurring ET-3 and ET_B mutations, respectively (38, 39). In humans, multigenic Hirschsprung disease is caused by mutations in ET_B and ET-3 genes (49–51). Although these gene knockout mice have highlighted the critical roles of endothelins in development, this has postponed the development of adult animal models lacking endothelin system components.

Endothelins in the Vessels

Since its original discovery as a vasoconstrictive peptide, recognition of the role of ET-1 in vascular homeostasis has been firmly established and further extended to several vessel pathologies. The only vascular endothelin identified is ET-1. It is secreted by endothelial cells and, in inflammatory states, by vascular smooth muscle cells (3, 52). The local ET-1 concentration within vascular wall is ≥100-fold that of plasma level, stemming in part from the fact that 80% of ET-1 is secreted on the basal side of endothelial cells (53, 54). ET-1 interacts with ET_A found on underlying smooth muscle cells as well as ET_B found on endothelial cells and on some smooth muscle cells (10, 11, 55). The ratio of the two receptors on smooth muscle cells varies depending on the vascular bed, with veins in general having lower ET_A: ET_B ratios than arteries (56). The level of ET_B expression on smooth muscle cells can also be increased in vascular pathologies (55).

Intravenous bolus administration of ET-1 in different species leads to a short-lived decrease (up to a few minutes) in vascular resistance, followed by the long-term (≤1 h) increase, implicating a balancing act of dilator and pressor functions for endothelins (57). The initial increase in perfusion is caused via nitric oxide and prostacyclin release by ET_B-stimulated endothelial cells, because inhibitors of prostacyclin and nitric oxide synthesis or ET_B antagonists have been shown to abolish the dilator response both in vivo and in vitro (58, 59). The decrease in perfusion is primarily mediated by ET_A on smooth muscle cells, as shown by

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the ability of the ET_A antagonists to greatly attenuate the pressor response (60). Further evidence of endogenous endothelin's involvement in basal vascular-tone regulation comes from experiments in which specific endothelin system components are blocked (61). Administration of the ET_A-selective antagonist BQ-123 into the human forearm vasculature leads to an increase in local blood flow (62). A >10-mm Hg decrease in systemic blood pressure is noted in humans upon administration of the ET_A/ET_B-combined antagonist TAK-044 (63). Similar hypotensive effects are noted when ET_A antagonists are administered to guinea pigs and ECE inhibitors are administered to rats (64, 65). However, not all studies replicate the blood pressure-lowering effect of ET_A blockade in normotensive animals (66). Genetic evidence for the direct role of ET-1 in blood pressure regulation comes from experiments using endothelial-cell-specific ET-1 knockout mice. Mice lacking ET-1 in endothelial cells have a 15-mm Hg reduction in mean, systolic, and diastolic blood pressures in comparison to the genetically matched control groups. The blood pressure response to inhibitors of the angiotensin and sympathetic systems is otherwise the same in all groups, implying that the hypotensive effect does not directly involve these regulatory systems but that it is mediated in the vessel wall by the interaction of endothelial cells with smooth muscle cells via ET-1 (23). A more complete understanding of the interplay of the endothelin system components in the vasculature awaits tissue-specific targeting of ET_A in smooth muscle cells and ET_B in endothelial cells.

The question of endothelins' involvement in hypertension has been an important issue since their identification. Most patients with hypertension do not have pharmacologically active concentrations of plasma endothelins (37, 67). The exception is provided by two cases of hemangioendothelioma that presented with markedly elevated high levels of plasma ET-1 and high blood pressure. Removal of the tumors led to normalization of the circulating ET-1 level and blood pressure (68). However, ET-1 dysregulation in the local environment could still be involved in high blood pressure, because hypertensive patients with renal failure or vascular disease show a ≤10-fold elevation in plasma ET-1 (37). This finding suggests decreased local clearance and/or increased local production of ET-1 in these types of hypertension. The diastolic blood pressure in humans with essential hypertension, when treated for 4 weeks with the ET_A/ET_B-combined antagonist bosentan, was lowered by 5.7 mm Hg, a reduction comparable to that of losartan, the AT1 angiotensin II receptor antagonist (69). The treatment did not result in activation of the sympathetic nervous system or the renin-angiotensin system. Additional proof for endothelins' role in hypertension can be derived from experiments on rat models of salt-dependent hypertension. The high blood pressures in the deoxycorticosterone acetate salt- and the Dahl salt-sensitive rats are particularly well treated by ET_A antagonism (70, 71). However, the roles of the endothelins in human hypertension still remain to be clearly defined.

Besides participating in the regulation of vascular tone, the endothelin system has also been implicated in a number of vascular pathophysiologies, including post-subarachnoid hemorrhage cerebral vasospasm and atherosclerosis. In cerebral

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vasospasm, inflammation-induced release of ET-1 leads to massive constriction of blood vessels supplying the brain. Higher plasma levels of ET-1 are reported in patients suffering from subarachnoid hemorrhage with cerebral vasospasms (72). A better clinical outcome was reported when ET_A was antagonized in animal models of cerebral vasospasm (73). When administered intracisternally, the ET_A-selective antagonist BQ-123 attenuates the cerebral vasoconstriction after induced subarachnoid hemorrhage in rats. In a canine model of subarachnoid hemorrhage, the combined ET_A/ET_B antagonist bosentan attenuates by half the decrease in basilar artery diameter (74).

The endothelin system also plays a role in atherosclerosis. In this pathology, endothelial cells and macrophages release ET-1 that activates ET_A found on macrophages, smooth muscle cells, and fibroblasts. ET-1 has mitogenic effects on smooth muscle cells and fibroblasts, and it stimulates synthesis of inflammatory mediators in macrophages and fibronectin in smooth muscle cells (75–78). Treatment with oxidized low-density lipoproteins, a well-known atherogenic factor, stimulates ET-1 synthesis in human endothelial cells and macrophages (79, 80). The tissue ET-1 level is increased up to sixfold in atherosclerotic aortic arches of rabbits fed a high-cholesterol diet (81). The presence of endothelin receptors in atherosclerotic plaques has also been confirmed. When administered intravenously, radiolabeled ET-1 accumulates within the atherosclerotic plaques, and binding sites for ET-1 are detected within hyperplastic lesions in both human and porcine blood vessels (82, 83). A beneficial role of ET_A antagonism in delaying the development of atherosclerosis is shown by many studies. In cholesterol-fed hamsters, the ET_A-selective antagonist BMS-182874 decreases the surface of aortic fatty streaks and the number of macrophages in vessel walls by 80% (84). When apolipoprotein E-deficient mice fed a high-fat and -cholesterol diet are treated with the ET_A-selective antagonist LU135252, they exhibit a normal response to nitric oxide and a 30% reduction in plaque size. Blood pressure or cholesterol level is not changed by the treatment, implying that the beneficial outcome is due to effects in the vasculature (85). Additionally, the apolipoprotein E-deficient and low-density-lipoprotein-deficient mice fed "Western" diets develop coronary atherosclerosis, and, when subjected to mental stress or hypoxia, they progress to acute myocardial ischemia. The acute administration of the ET_A-selective antagonist LU135252 both before and right after the stressful event attenuates the electrocardiogram changes and the troponin T elevation that were observed in the nontreated group. This implies that ET_A receptor signaling mediates the ischemic response to stress in the atherosclerotic vessels of the heart (86).

Endothelins in the Heart

The endothelin system participates in both cardiac physiology and pathology. ET-1, the predominant cardiac endothelin, is made by cardiomyocytes, endothelial cells, and cardiac fibroblasts (3, 15, 87). Both the ET_A and ET_B receptors are found on

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cardiomyocytes and fibroblasts, with the ET_A receptor representing 90% of endothelin receptors on cardiomyocytes (15, 88). Additionally, ET_A and ET_B receptors are found on smooth muscle cells and endothelial cells of the coronary vessels.

The endothelin system has inotropic and chronotropic effects on heart explants. Nanomolar concentrations of ET-1 have a positive inotropic effect on the isolated, stimulated left atria of guinea pigs (89). There is a dose-dependent increase by ET-1 in the rate of sinoatrial-node firing in the isolated right atria of guinea pigs (90). However, in isolated guinea pig myocyte cultures, ET-1 induces hyperpolarization and shortens action potential duration, implying that, under certain conditions, the endothelin system has chronotropic and inotropic effects in the heart (91, 92). The discrepancy can be most easily explained by desensitization of ET_A receptors involved in negative chronotropic effects in some experimental models (93). In vivo, the significance of endothelins in inotropy or chronotropy is even more elusive, because pharmacological interventions directly affect the closely linked coronary vascular function (94). In one experimental setup, the ET_A-selective antagonist BQ-123 administered directly into the human coronary artery decreases left-ventricular pressure changes without altering right-atrial or pulmonary pressure (95). This result shows that the endothelin system may have a positive basal inotropic effect in the human heart. However, further in vivo study of cardiac physiology is necessary to clearly outline the role of endothelins in chronotropy and inotropy.

Besides the proposed causative role in atherosclerosis leading to cardiac ischemia, the endothelin system is also involved in the pathophysiology of myocardial infarction and congestive heart failure. As demonstrated by the administration of ET-1 into the porcine coronary circulatory system, the coronary vessels react to the peptide by vasoconstricting, leading to ischemia and ventricular arrhythmias (96). The endothelin system is altered in severe cardiac ischemia, because plasma ET-1 concentrations are elevated >fivefold within a few hours of human myocardial infarct (97). Antagonism of the endothelin system is generally beneficial in cardiac ischemia. In dogs with induced myocardial infarcts, the ET_A-selective antagonist BQ-123 administered during a 90-min period of coronary artery blockage decreases the infarct size by $\leq$40% (98). The effect is not attributed to changes in the peripheral hemodynamics but instead either to an inhibition of toxic effects of endothelin on cardiomyocytes or to the prevention of cardiac ischemia through a dilation of collateral vessels.

In postinfarct states, the endothelin system appears to have dual roles: a beneficial one in tissue repair and restoration of cardiac function and a deleterious one in development of congestive heart failure. The plasma ET-1 level is elevated in patients with congestive heart failure, and their cardiac tissues have increased levels of ET-1 and ET_A (99–101). ET-1 has a potent hypertrophic effect on cultured rat neonatal cardiomyocytes, suggesting that it may have a role in remodeling of the adult heart. Activation of ET receptors leads to an induction of markers of cardiac remodeling, including mRNAs for troponin I, myosin light-chain 2, and skeletal

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alpha actin (102). A number of published reports have shown a beneficial effect of ET_A antagonism in congestive heart failure models. Twelve-week treatment with the ET_A-selective antagonist BQ-123 increases about twofold the survival rate of rats when the antagonist is administered 1 week after coronary artery ligation-induced myocardial infarction (15). Treatment with BQ-123 decreases pulmonary pressure and prevents an increase in ventricular mass and cardiac remodeling, while having no effect on systemic arterial pressure. A 9-month treatment with the combined ET_A/ET_B antagonist bosentan leads to a similar improvement in survival rate (65% in the treated group vs 47% in the control group) of rats with congestive heart failure induced by myocardial infarction (103). Treatment with bosentan attenuates cardiac remodeling and hypertrophy and blunts the increase in pulmonary and aortic pressures. It is not clear whether these effects are caused by a direct effect on cardiomyocytes, a reduction of the after load and/or preload on the ventricles of the heart, or some other factors. Implying the counteracting roles of the two receptors in congestive heart failure, the ET_B-selective antagonist RES-701-1 has an unfavorable effect on cardiovascular parameters of dogs with induced congestive heart failure (104). Treatment with this ET_B antagonist leads to increased left ventricular pressure and decreased cardiac output. A detrimental effect or lack of a beneficial one is observed after cardiac ischemia, when the ET_A antagonists are given immediately after the ischemic episode. When rats are treated with the ET_A-selective antagonist EMD 94246 starting on the day of induced myocardial infarction, they exhibit no changes in hemodynamic parameters or survival over the 8 weeks of study (105). Similarly, the administration of ET_A-selective antagonist LU135252 to rats with congestive heart failure 1 day after induced myocardial infarction resulted in thinning of the left ventricular scar, dilation of the left ventricle, and no effect on survival rate (106). Since endothelin receptors are present on fibroblasts and macrophages, two participants in the myocardial-tissue repair process, it is likely that the endothelin system has a beneficial role immediately after a myocardial infarct. Current observations indicate beneficial effects of endothelin receptor activation until up to a few days after the myocardial infarction and long-term deleterious effects during development of congestive heart failure afterwards.

Acute treatment with endothelin antagonists in patients with congestive heart failure shows the beneficial hemodynamic effects of such regimens. The combined ET_A/ET_B antagonist bosentan was given acutely to patients with stable congestive heart failure who were taken off their normal drug treatment (107). Compared with placebo, bosentan reduced pulmonary and mean arterial pressures by ~10%, decreased pulmonary and systemic resistance by 30 and 15%, respectively, and increased the cardiac index by 13%. Similar benefits were seen in patients with stable congestive heart failure who were given the ET_A-selective antagonist BQ-123 in addition to their normal medication (107). The treatment led to decreased pulmonary and arterial pressures, decreased systemic but not pulmonary resistance, and an increased cardiac index (108). More studies, particularly involving chronic treatments, are warranted to establish the effects of endothelin antagonism in patients.

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Endothelins in the Lung

In addition to their presence in pulmonary vessels, endothelin system components are found in the airways, where they have been implicated in pulmonary hypertension and chronic airway inflammation. In the lung, ET-1 is expressed by endothelial cells, airway epithelial cells, and macrophages (16, 17). Endothelin receptors are present on airway smooth muscle cells and, in a typical fashion, on the vessels of pulmonary circulation. The ratio of airway endothelin receptors is species dependent. In human bronchi, the ET_B receptor is tenfold more abundant than the ET_A receptor, whereas the reverse is true in sheep bronchi (109–111). In rodents, the airway receptors are present in approximately equal amounts (112). ET_B receptors are also found on neuronal bodies and on neuronal processes of the intramural tracheal autonomous nervous system (113).

The endothelin system influences airway tone. In vivo, in a guinea pig model of lung performance, the intravenous administration of ET-1 increases airway resistance by up to fourfold in a dose-dependent manner (114). This result highlights the role of ET-1 as a potent airway constrictor, which may have a particular importance in the pathogenesis of interstitial lung diseases and asthma. Genetic evidence shows that mice overexpressing ET-1 in the lungs have progressive pulmonary fibrosis and accumulation of inflammatory cells without pulmonary hypertension, implying a role of endothelins in pulmonary remodeling (115). The role of endothelins in interstitial lung disease is also illustrated by an in vivo study in which ET-1 was overexpressed in alveolar macrophages, bronchial epithelium, and alveolar epithelium, using hemagglutinating virus of Japan liposome-mediated gene transfer (116). Rats that overexpress ET-1, excluding the control animals exposed to the viral vector alone, show edematous alveolar septa and hyperplasic connective tissue plaques. The histology is virtually identical to human broncholitis obliterans. In asthma, the ET_A-selective antagonist BQ-123 blocks the immediate and late phase of asthmatic response in a guinea pig model (117). Additionally, patients with asthma have high levels of ET-1 in bronchoalveolar lavage fluid (118), and, when they are challenged with aerosolized ET-1, they respond with dose-dependent bronchoconstriction that is not seen in normal volunteers (119).

In the lung vasculature, ET-1 induces vasoconstriction, which can be blocked by ET_A antagonists (120). This vasoconstriction is preceded by a short period of vasodilation, as observed in blood-perfused rat lungs (121). However, the response is also influenced by the basal tone of vessels, because infusion of ET-1 during hypoxia results in overall pulmonary dilation (122). Patients with pulmonary hypertension have increased levels of ET-1 mRNA and ET-1 peptide in the endothelial cells of pulmonary arteries (123). Unlike normal subjects, patients with pulmonary hypertension have higher pulmonary arterial vs venous plasma ET-1 levels, implying increased pulmonary ET-1 production combined with its decreased lung clearance (124). In various animal models, ET_A antagonism can block progression of the disease. In rats with monocrotaline-induced pulmonary hypertension, continuous infusion of the ET_A-selective antagonist BQ-123 inhibits the increase

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in pulmonary pressure (125). This treatment also prevents right-ventricular hypertrophy and pulmonary artery medial thickening. In a dog model of pulmonary hypertension, the ET_A-selective antagonist FR139317 reduces pulmonary vascular resistance and mean pulmonary arterial pressure (126). In contrast, the ET_B-selective antagonist RES-701-1 administered to these dogs leads to an increased pulmonary vascular resistance and pressure. These findings point out the divergent roles of ET_A and ET_B in the development of pulmonary hypertension and the beneficial effect of ET_A antagonism on progression of the disease.

Endothelins in the Kidney

The endothelin system is intimately involved in both renal physiology and pathology. ET-1 is synthesized by vessel endothelial cells, and ET-1 and ET-3 are made in various nephron cells, including the epithelial cells of medullary and cortical collecting ducts (18, 19). Both ET_A and ET_B are present in renal vessels and on tubular epithelial cells, where ET_B is the predominant receptor type. The highest density of ET_B is seen on the epithelial cells of the inner medullary collecting duct (127, 128).

The endothelin system controls renal blood flow, reabsorption of water and sodium, and acid-base balance. The effect on renal vessels is demonstrated by ET-1 administration into the renal artery in anesthetized rabbits (129). The treatment reduces renal blood flow, cortical perfusion, glomerular-filtration rate, urinary flow, and sodium excretion. The opposite effect is observed deep in the medulla, where the activation of ET_B causes natriuresis and diuresis. Big ET-1, when administered into the femoral artery of rats, produces a significant increase in sodium and water excretion that cannot be blocked by the ET_A-selective antagonist BQ-123 (130). The diuresis and natriuresis are explained by activation of ET_B by ET-1 that is generated from big ET-1 at the level of the medullary collecting duct. The mechanism of diuresis is linked to natriuresis and to an endothelin-mediated inhibition of vasopressin's action in the inner medullary collecting duct. As demonstrated in a cell culture of inner medullary collecting epithelium, ET-1 is able to lower water permeability sixfold in vasopressin-stimulated cells (131). In vivo, this would reduce water reuptake in the duct and increase urinary water loss. The role of endothelins in sodium excretion and acid-base status has been further explored in mice and rats genetically lacking ET_B (132, 133). These rodents are rescued from lethal intestinal obstruction by transgene-driven expression of ET_B to the enteric nervous system precursors of the ET_B-null animals. These otherwise ET_B-deficient animals exhibit significant hypertension that is induced by a sodium chloride-containing diet. The markedly salt-sensitive hypertension is caused neither by alteration of nitric oxide and prostacyclin production nor by augmentation of ET_A signaling, because administration of N(G)-L-arginine-methyl-ester, indomethacin, and the ET_A-selective antagonist FR139317 did not correct the difference in blood pressures. These data suggest that ET_B likely acts within the kidney, not in the vessel wall, to regulate blood pressure. The hypertension is also independent of the renin-angiotensin system, since captopril, a blocker of angiotensin II production, is

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not able to correct the high blood pressure. The luminal epithelial sodium channel (ENaC) blocker amilioride restores normal mean arterial pressure in the salt-loaded ET_B-deficient animals, but it has no effect on the wild-type controls (134, 135), which suggests that in vivo ET_B in the collecting duct tonically inhibits the activity of ENaC, the final regulator of sodium balance.

The endothelin system, through ET_B, plays a role in renal acid-base regulation. Mice lacking ET_B in the kidney, while maintaining normal blood acid-base status under baseline conditions, have low levels of urinary ammonium and high levels of urinary phosphate and citrate (136). When challenged with acid ingestion, ET_B-deficient mice develop more severe metabolic acidosis than the wild-type controls, which is at least partially due to lack of stimulation of the proximal tubule apical membrane Na^+/H^+ (NHE3) antiporter upon the acid challenge (137). Wild-type rats, when challenged with an acid diet, increase their acid excretion by, among other mechanisms, elevating expression of NHE3 in the brush-border membranes of the proximal tubule and thick ascending limb (138). In cells expressing the NHE3 antiporter, ET_B receptor activation leads to greater proton transport (139). All of these observations point out the ET_B-mediated stimulatory role of endothelins in proton secretion in the proximal tubule.

The endothelin system has been implicated in renal pathology. Endothelins have a role in renal remodeling, as shown by overexpression of human ET-1 in mice (140). Despite normal blood pressure, the transgenic mice show interstitial fibrosis, glomerulosclerosis, and declining renal function. Endothelins play an active role in the progression of renal failure. The extent of chronic renal failure directly correlates with the renal ET-1 level in rats (141). In partially nephrectomized rats, a model of progressive renal disease, infusion of the ET_A receptor antagonist FR 139317 beginning 1 week after surgery lowers proteinuria and prolongs animal survival (142). In rats with acute renal failure induced by 1-h ischemia, the ET_A-selective antagonist BQ-123 improves glomerular filtration rate and net tubular reabsorption, two indices of kidney function (143). In similar experiments, the ET_A-selective antagonist BQ-123 also improves the survival of rats with induced acute renal failure, likely owing to proper maintenance of the plasma potassium levels (144). A decrease in accumulation of macrophages and myofibroblasts is observed in the rats with acute renal failure that are treated with the ET_A antagonist PD156707, suggesting a protective effect mediated by reduction of these inflammatory cells in the kidney (145). These findings suggest a role for endothelins in both kidney physiology and pathology, but the exact molecular mechanisms of renal protection by endothelin antagonism awaits further studies.

Endothelins in the Brain

The endothelin system is widespread in the brain, suggesting a variety of possible functions. ET_A, ET_B, ET-1, and ET-3 are expressed in the brain by vascular, neuronal, and glial cells. Endothelin immunoreactivity is present in neurons of the cerebral cortex, striatum, amygdala, hippocampus, paraventricular and supraoptic

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nuclei of the hypothalamus, subfornical organ, median eminence, raphe nuclei, and pituitary gland (20–22). ET-1 is the predominant neural endothelin, except for a predominance of ET-3 in the pituitary gland (24, 146). ET_A is immunohistochemically identified in the neurons of A1–A7 noradrenergic loci, in A8–A17 loci, and in a magnocellular subset of the supraoptic and paraventricular hypothalamus. This localization correlates with c-*fos* expression induced by central injection of ET-1 (147). ET_B is immunohistochemically localized to the neurons of the diagonal band of Broca, the fibers of organum vasculosum of lamina terminalis, the fibers of median eminence, and the thick fibers of hypothalamic neurons (148), which are immunoreactive with luteinizing hormone-releasing hormone. Both ECE-1 and ECE-2 are also found in the brain (5–7).

The expression of endothelin system components in many discrete areas of the brain suggests a wide array of functions. Intraventricular injection of ET-1 results in transient increases (up to several minutes) in heart rate, arterial pressure, renal sympathetic nerve activity, and respiratory rate. High doses of ET-1 first lead to this transient increase, then cause long-term depression of these parameters (149). The change in arterial blood pressure is mediated by stimulation of the sympathetic outflow, because the treatment with an alpha-1 adrenergic blocker abolishes the ET-1 effect on blood pressure (150). These changes are caused by a direct effect on neurons or glial cells, because the regional blood flow is not immediately affected by high-dose intraventricular administration (151). The effects of cerebroventricular ET-1 administration can be replicated by microapplication of ET-1 to specific sites involved in central respiratory and circulatory regulation, such as ventrolateral medulla and the nucleus tractus solitarius (152, 153). Also, because the effect on the ventrolateral medulla is blocked by the ET_A-specific antagonist BQ-123 but not by the ET_B-selective antagonist BQ-788, the ET_A receptor must be involved in signal transduction in this area (154). Genetic evidence also demonstrates the importance of the endothelin system in central blood pressure regulation. ET-1 knockout-heterozygous mice exhibit a 10-mm Hg increase in mean, systolic, and diastolic blood pressures (42). This increase is perplexing, because theoretically a reduction in the levels of vasoconstrictive peptide should result in lower blood pressure. This increased blood pressure has been attributed to the greater sympathetic nervous system output, because these artificially ventilated, urethane-anesthetized mice exhibit increased renal sympathetic nerve activity (155). Heterozygous ET-1-deficient mice generated in another laboratory, however, did not exhibit the increased blood pressure when measured by the same catheterization procedures (23). The discrepancy is most easily explained by the difference in genetic backgrounds between the two groups of knockout mice.

The critical role of endothelins in respiratory control is pinpointed by several experiments. In neonatal pigs, intracisternal injection of ET-1 induces apnea, whereas direct injection into the ventrolateral medulla induces complete cessation of respiratory activity (156). ET-1 knockout mice exhibit some unusual respiratory characteristics in comparison with wild-type mice. Heterozygous animals have lower arterial oxygen concentrations and higher arterial carbon dioxide concentrations

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but maintain a normal respiratory rate and volume. In a hypercapnic or hypoxic environment, these mice have attenuated physiological increases in respiratory volume and rate (157). The homozygous ET-1-deficient neonates, when cesarean-section delivered and tracheotomized, have normal ventilatory volume in room air, but they exhibit a lack of the normal increase in ventilatory volume when placed in hypoxic or hypercapnic conditions (41). Medulla oblongata preparations from ET-1-deficient neonates do not show a normal increase in the discharge rate at lower pH, the response observed in the wild-type preparations (158).

Besides regulation of the circulatory and respiratory functions, endothelins are implicated in control of the release of neuropeptides from the pituitary and hypothalamus. In rat primary pituitary cell cultures, ET-3 inhibits prolactin release whereas the ET_A-specific antagonist BQ-123 increases that release (159). In the same cell cultures, ET-3 and ET-1 stimulate the release of luteinizing hormone and follicle-stimulating hormone, while ET-3 stimulates the release of thyroid-stimulating hormone (160). In rat hypothalamic explants, ET-1 stimulates vasopressin and oxytocin release that is blocked by the ET_A-specific antagonist BQ-123, whereas ET-1 has no effect on the level of corticotropin-releasing hormone (161).

The endothelin system is also implicated in modulation of behavior and metabolism. Intraventricular injection of ET-1 in rats results in a gamut of behavioral changes, including barrel rolling, body tilting, nystagmus, facial clonus, forelimb clonus, and tail extension, most effects at doses that do not cause any changes in cerebral blood flow (162). Injection of ET-1 into the periaqueductal gray matter reduces pain response in mice subjected to the hot plate paradigm in a dose-dependent manner (163). These data imply that endothelin has a role in neurotransmission that is important for an animal's proprioception. Other studies implicate endothelin involvement in control of fluid intake and energy expenditure. Even though ET-3 has no direct effect on drinking, its administration suppresses water intake in animals that are water deprived or treated with angiotensin II (164, 165). Intracerebroventricular injection of ET-3 decreases the core temperature of rats by 1°C over a period of 2 h (165). Better understanding of these pharmacologically implied roles of endothelins will come from animals with altered expression of the ET system components in a brain region-specific-manner.

SUMMARY

Endothelins can be considered stress-responsive regulators working in paracrine and autocrine fashion in a variety of organs, with both beneficial and detrimental roles in mammals (Table 3). In the vessels, the endothelin system has a basal vasoconstricting role and participates in development of atherosclerosis and subarachnoid hemorrhage. In the heart, it affects inotropy and chronotropy, and it mediates cardiac remodeling in congestive heart failure. In the lungs, the endothelin system regulates the tone of pulmonary airways and vessels and is involved

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TABLE 3 Proposed in vivo functions of the endothelin system[a]

Function(s)	Endothelin components involved
Beneficial roles	
Embryonic and neonatal development	
Cephalic and cardiac neural crests	ET-1, ECE-1, ET_A
Enteric neurons and skin melanocytes	ET-3, ECE-1, ET_B
Neonatal growth and intestinal functions	ET-2, ECE-1(?), ET_A and ET_B(?)
Renal homeostasis	
Salt handling	ET-1, ECE-1(?), ET_B
Acid-base balance	ET-1, ECE-1(?), ET_B
Maintenance of basal vascular tone	ET-1, ECE-1(?), mostly ET_A
Cardiac tissue repair after acute ischemia	ET-1, ECE-1(?), mostly ET_A
Central respiratory regulation	Mostly ET-1, ECE-1 and ECE-2(?), mostly ET_A
Detrimental roles (in multiple tissues)	
Pathophysiological conditions involving abnormally elevated vascular tone	ET-1, mostly ECE-1, mostly ET_A
Chronic tissue damage/remodeling/ inflammatory responses	ET-1, mostly ECE-1, ET_A and ET_B

[a](?) = suggested involvement of these endothelin components.

in development of pulmonary hypertension. In the kidney, it controls water and sodium excretion and acid-base balance, and it participates in the pathophysiology of acute and chronic renal failure. In the brain, the endothelin system modulates cardiorespiratory centers and release of hormones. In addition to the organs described in this review, endothelins affect the physiology and pathophysiology of the liver, muscle, bones, skin, prostate, adipose tissue, and reproductive tract (26). The diverse expression pattern of the molecular components is further complicated by the complex pharmacology and often counteracting physiological actions. One classical example is the vessels, in which endothelium-derived ET-1 leads to both constriction via smooth muscle cell ET_A and dilation via endothelial ET_B, which shows the important role these peptides play as fine tuners of vascular tone. Similar "ying-yang" interactions probably take place in many other sites that express both endothelin receptors in the same cell type or within the same organ.

A further understanding of endothelin physiology depends on the ability of researchers to dissect the role of individual components of the endothelin system in specific organs or tissues. Development of new animal models, whether by cell population-specific deletion of a gene via conditional gene knockout or by specific overexpression of a gene in a pharmacologically inducible transgenic animal, will allow for examination of specific, localized actions of endothelins. The conditional gene knockout is particularly well suited for such studies, because generation of appropriate animal models overcomes not only the lethality of whole-animal gene

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deletion but also involvement of endothelins in related physiologies. The future use of these techniques will allow for clear genetic dissection of physiological and pathophysiological roles of the endothelin system and will lead to the knowledge necessary for more efficient identification of pharmacological targets.

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