*Adv Pharmacol*. Author manuscript; available in PMC 2013 June 26.

Published in final edited form as:
*Adv Pharmacol*. 2010 ; 60: 1–26. doi:10.1016/B978-0-12-385061-4.00001-5.

# The Cardiovascular Physiology and Pharmacology of Endothelin-1

**Eric Thorin**[*] and **Martine Clozel**[†]

[*]Department of Surgery, Montreal Heart Institute, Research Center, Université de Montréal, Montreal, Quebec, Canada [†]Actelion Pharmaceuticals Ltd., Allschwil, Switzerland

## Abstract

One year after the discovery in 1980 that the endothelium was obligatory for acetylcholine to relax isolated arteries, it was clearly shown that the endothelium could also promote contraction. In 1988, Dr Yanagisawa's group identified endothelin-1 (ET-1) as the first endothelium-derived contracting factor. The circulating levels of this short (21 amino acids) peptide were quickly determined in humans and it was reported that in most cardiovascular diseases, circulating levels of ET-1 were increased and ET-1 was then recognized as a likely mediator of pathological vasoconstriction in human. The discovery of two receptor subtypes in 1990, $ET_A$ and $ET_B$, permitted optimization of bosentan, which entered clinical development in 1993, and was offered to patients with pulmonary arterial hypertension in 2001. In this report, we discuss the physiological and pathophysiological role of endothelium-derived ET-1, the pharmacology of its two receptors, focusing on the regulation of the vascular tone and as much as possible in humans. The coronary bed will be used as a running example, but references to the pulmonary, cerebral, and renal circulation will also be made. Many of the cardiovascular complications associated with aging and cardiovascular risk factors are initially attributable, at least in part, to endothelial dysfunction, particularly dysregulation of the vascular function associated with an imbalance in the close interdependence of NO and ET-1, in which the implication of the $ET_B$ receptor may be central.

## I. Introduction

The endothelium is an extraordinary organ that protects the arterial wall through the release of nitric oxide (NO) and prostacyclin ($PGI_2$) among other factors (Furchgott & Zawadzki, 1980; Palmer et al., 1987). Before 1980, it was merely considered an inert barrier (Aird, 2007). The presence of an endothelium-derived constricting factor (EDCF) was hypothesized 1 year after the revelation of the relaxant properties of the endothelium (De Mey & Vanhoutte, 1982; Vanhoutte et al., 1986). But it was only 8 years later that Yanagisawa and colleagues identified the peptide endothelin-1 (ET-1) has this long-lasting EDCF (Yanagisawa et al., 1988a, 1988b). Two receptors for ET-1 were identified 2 years later (Arai et al., 1990; Sakurai et al., 1990). Then, shortly after the discovery of $ET_A$ and

*Conflict of Interest:* Dr. Martine Clozel is Chief Scientific Officer at Actelion Pharmaceuticals.

$ET_B$ receptors, Martine Clozel and colleagues presented in 1993 the first orally active ET-1 receptor antagonist, Ro 46-2005 (Clozel et al., 1993), and the same team made a structurally modified analog, bosentan (Tracleer), available to patients with pulmonary arterial hypertension (PAH) at the end of 2001. In less than 15 years, a new factor was identified, its receptors were cloned and their pharmacology characterized, a pathology associated with the abnormal function of the ET-1 system, and an effective treatment offered to patients in need. Today, other ET receptor antagonists have been synthesized and are in development, all this being well reviewed recently (Kirkby et al., 2008; Motte et al., 2006). There is still much to be discovered on the role and the mechanisms of action of ET-1. We focus in this chapter on the pharmacology of ET-1 and review the role of ET-1 on the vasculature with as much as possible references to the human pathophysiology.

## II. Cardiovascular Physiology of ET-1

ET-1 is one of the most potent vasoconstrictors identified so far (Yanagisawa et al., 1988b) inducing prolonged contraction of isolated canine and nonhuman primate coronary arteries with a half maximal effective concentration ($-\log[EC_{50}]$) of 8. The potency of ET-1 is unequaled, with the exception of urotensin II ($-\log[EC_{50}] = 9.5$; Douglas et al., 2000). ET-1 elicits its effects through two receptors (http://www.iuphar.org/): $ET_A$ receptors, located in vascular smooth muscle cells (VSMC) and cardiomyocytes, mediate contraction, whereas $ET_B$ receptors, located on vascular endothelial cells (EC), mediate dilation and ET-1 uptake, and regulate ET-1 production (Arai et al., 1990; Barton & Yanagisawa, 2008; Brunner et al., 2006; Callera et al., 2007; Dupuis et al., 1997; Farhat et al., 2008; Komukai et al., 2010; Rubanyi & Polokoff, 1994; Sakurai et al., 1990; Sanchez et al., 2002). Additionally, $ET_B$ receptors can also be expressed on VSMC and elicit contractions (Sanchez et al., 2002; Teerlink et al., 1994). It is a known fact that ET-1 is released continuously, mostly from EC, by a constitutive pathway and contributes to the regulation of the vascular tone in general (Brunner et al., 2006; Callera et al., 2007; Rubanyi & Polokoff, 1994). NO, however, strongly inhibits the release of ET-1 from the native endothelium (Boulanger & Luscher, 1990; Luscher et al., 1990); for this reason, it has been suggested that NO and ET-1 regulate each other through an autocrine feedback loop (Alonso & Radomski, 2003; Luscher et al., 1990). In addition to EC, ET-1 is also produced by VSMC, cardiomyocytes, leukocytes, macrophages, various neurons, and other cells (Kedzierski & Yanagisawa, 2001). This peptide is also proinflammatory and promotes VSMC proliferation (Anggrahini et al., 2009; Dashwood et al., 1998a; Davenport & Maguire, 2001; Ihling et al., 2001; Ivey et al., 2008; Ruschitzka et al., 2000). Thus, ET-1 contributes to the cardiovascular homeostasis by regulating basal vascular tone and remodeling (Brunner et al., 2006; Kedzierski & Yanagisawa, 2001).

### A. The ET-1 System

**1. Endothelins and Their Receptors—**There are three isoforms of endothelin produced in humans, ET-1, ET-2, and ET-3 (Inoue et al., 1989a; Saida et al., 1989). They are encoded on chromosomes 6, 1, and 20, respectively (Inoue et al., 1989a). ET-1 binds $ET_A$ and $ET_B$ receptors with equal affinity, while $ET_A$ receptors have approximately 100-fold less affinity for ET-3 than $ET_B$ receptors (Davenport, 2002). In addition, snake venom toxins called

"sarafotoxins" have been identified by homology. Sarafotoxin 6c (S6c) is a selective $ET_B$ receptor agonist (Rubanyi & Polokoff, 1994). $ET_A$ and $ET_B$ receptors belong to the 7-transmembrane domain (7-TM) family and are encoded by distinct genes on chromosomes 4 and 13, respectively (Sakurai et al., 1990).

ET-1 is produced within the cell in two proteolytic steps from the pre-proET-1, a large precursor peptide of approximately 200 amino acid residues. First, a furin-like neutral endopeptidase cleaves the preproET-1 to bigET-1, an inactive peptide of 41 amino acid residues (Denault et al., 1995; Laporte et al., 1993). Second, bigET-1 is cleaved by the endothelin-converting enzymes (ECE-1 and ECE-2) to the biologically active ET-1, a 21-amino acid residue peptide enclosed by disulfur bonds (Takahashi et al., 1993) mainly by EC (Inoue et al., 1989b). Some alternative pathways to ECE for the synthesis of ET-1 have been reported: a chymostatin-sensitive enzyme, such as chymase, and the matrix metalloproteinase 2 are able to convert bigET-1 to mature ET-1 in human blood vessels (Maguire & Davenport, 2004; Maguire et al., 2001).

The clearance of ET-1 from the circulation after an intravenous injection of radiolabeled ET-1 in rats is rapid (half-life of 40 s; Sirvio et al., 1990), while its pressor effect is long lasting (≈ 1 h at the doses administered) in man (Sirvio et al., 1990; Vierhapper et al., 1990). The majority of ET-1 is retained by the lungs and cleared from the circulation via binding to $ET_B$ receptors (Dupuis et al., 1996a, 1996b).

## 2. Endothelin Receptor Ligands and Pharmacology: Emerging Concepts—

There is no selective agonist for the $ET_A$ receptor. ET-1 [1–31] has been shown to have more selectivity for $ET_A$ compared to $ET_B$ receptors (Rossi et al., 2002), but the 31-amino acid peptide has no direct pharmacological effects if not converted via a neutral endopeptidase-dependent mechanism to ET-1 [1–21] (Fecteau et al., 2005). Selective antagonists for the $ET_A$ receptor include ZD4054, atrasentan, darusentan, macitentan, ambrisentan, and sitaxsentan (Motte et al., 2006).

In contrast to $ET_A$ receptors, selective $ET_B$ receptor agonists are available such as S6c and IRL-1620. Selective antagonists of the $ET_B$ receptors include BQ788, A192621, RES7011, and IRL2500 (Alexander et al., 2009).

Because ET receptors are 7-TM receptors, their signal transduction (Fig. 1) was first interpreted as a sequential series of events initiated by the binding of the agonist on its receptor. This simplistic view had to be revised with the evidence that activation of a 7-TM receptor can activate simultaneously multiple pathways (Watts, 2010) including some G-protein-independent pathways (Galandrin et al., 2007; Kenakin, 2007; Violin & Lefkowitz, 2007). One well-known example is the activation of the angiotensin II (ANG II) receptor ($AT_1$): this receptor activates both G-protein-dependent path-ways (PLC, PKC, channels, etc.) and β-arrestin-dependent pathways (independent of G proteins, i.e., the src/extracellular signal-regulated kinase/mitogen-activated protein kinase pathway). ANG II activates both signaling pathways; however, the substituted ANG II peptide $Sar^1$, $Ile^4$, $Ile^8$-ANG II (SII) almost exclusively activates the β-arrestin-dependent pathways (Violin & Lefkowitz, 2007). SII is called a "biased agonist" since it activates a preferential signal transduction pathway.

Other biased ligands to several 7-TM receptors have been discovered (Gesty-Palmer et al., 2009; Rajagopal et al., 2010; Violin & Lefkowitz, 2007) including the $\beta_2$-adrenergic receptor: carvedilol, a β-adrenergic receptor antagonist, is able to stabilize a receptor conformation, which, although uncoupled from $G_s$, is nonetheless able to stimulate β-arrestin-mediated signaling (Drake et al., 2008; Wisler et al., 2007). Through the activation of $ET_A$ receptors, ET-1 stimulates both G-protein-dependent and independent pathways (Rosano et al., 2009; Spinella et al., 2009). It is therefore almost obvious that ET-1 acts as a biased ligand. This is also true for $ET_B$ receptors, since ET-1 induces internalization of $ET_B$ receptors and G-protein-dependent pathways (Farhat et al., 2008; Spinella et al., 2009). Are there specific conditions necessary to reveal the biased activation of $ET_A$ by ET-1? What would be the conditions for ET-1 to act like carvedilol does on the β-adrenergic receptor, that is, solely activate the β-arrestin-mediated signaling? Are there pathological conditions that may affect ligand binding and signal transduction? This is an extremely important question because disease states or even aging alone could be responsible for changes in the microdomain such as lipid composition, influencing ligand binding, receptor dimerization (see later), and the subsequent signal transduction. It is known, for example, that oxidized low-density protein can change the microviscosity of the endothelium plasma membrane and alters signal transduction (Hamilton et al., 1994; Thorin et al., 1995). Much, however, needs to be understood from vascular primary cell cultures, isolated vessels, and *in vivo* preparations.

Another recent change in the pharmacological concepts of receptor signal transduction has been introduced by the evidence that ET receptors can form heterodimers, forcing us to change the way we interpreted pharmacological signals (Dai & Galligan, 2006; Evans & Walker, 2008a, 2008b; Gregan et al., 2004; Sauvageau et al., 2006). This observation, however, remains to be translated into physiological significance. So far, heterodimer formation has been reported in heterologous cell preparations expressing $ET_A$ and $ET_B$ receptors. To the best of our knowledge, we reported the only evidence of potential heterodimers expressed in rat pulmonary arteries (Sauvageau et al., 2006); in these vessels, the pharmacology is complex and difficult to interpret using the classical pharmacological concept of sequential events since for the least, cooperation between the two receptor subtypes exists. In addition, the pharmacology of ET receptors changes in pathological conditions such as experimental pulmonary hypertension in rats (Sauvageau et al., 2009). Although heterodimer formation of ET receptors is likely, the challenge will be to characterize their functions. Another level of complexity has been recently reached with the report that $ET_B$ and dopamine $D_3$ receptor heterodimerization (Yu et al., 2009; Zeng et al., 2008): the authors reported aberrant interactions between these two receptors in cultured renal proximal tubule cells with basal $D_3/ET_B$ receptor coimmunoprecipitation three times greater in Wistar Kyoto rats (WKY) than in spontaneously hypertensive rats (SHR). *In vivo*, the $D_3$ receptor agonist PD128907 caused natriuresis in WKY, which was partially blocked by $ET_B$ receptor antagonism. In contrast, PD128907 blunted sodium excretion in SHR. The authors therefore speculated that there was interaction between the two receptors and that these heterodimers could be partly responsible for hypertension in SHR. If these results can be confirmed in other settings, it will only confirm the complexity of the ET-1 system and offer alternative explanations to unexplained effects of ET-1 in the various systems tested.

When trying to conceive a biological path linking two receptors as dimers, one cannot exclude the possibility that ET-1 is a bivalent ligand, capable of stimulating both receptors simultaneously and thus promoting dimerization of the receptors. This possibility was first proposed by Himeno and collaborators (Himeno et al., 1998), and it was based on the following observation: selective $ET_B$ receptor ligands such as S6c, IRL1620, and BQ-788 competitively inhibited $^{125}$I-ET-1 binding only when BQ-123 (selective $ET_A$ receptor antagonist) was present in the incubation buffer. This therefore suggests that the $ET_B$ receptor is capable of binding ET-1 when the $ET_A$ receptor is being occupied by BQ-123. A collaboration mechanism between the $ET_A$ and the $ET_B$ receptors may function in the recognition of ET-1, which is the qualification of a typical “bivalent” ligand. This could be at the basis of the formation of heterodimers at the surface of VSMC (Harada et al., 2002). Unfortunately, no other studies are available that could support this possibility.

Finally, ET-1 can bind with high affinity a newly identified atypical rat receptor, the dual ET-1/ANG II receptor (DEAR) and induces a rise in intra-cellular $Ca^{2+}$ as efficiently as does ANG II (Ruiz-Opazo et al., 1998). The *Dear* gene maps to rat chromosome 2 and cosegregates with blood pressure in female F2(normotensive × hypertensive and salt-sensitive [R × S]) intercross rats with highly significant linkage (LOD 3.61) accounting for 14% of blood pressure variance. In *Dear*$^{-/-}$ mice, angiogenesis is impaired, the neuroepithelial development dysregulated, and is lethal by embryonic day 12.5 (Herrera et al., 2005). Interestingly, mouse DEAR does not bind ANG II as the rat DEAR does, but binds ET-1 and the vascular endothelial growth factor (VEGF) signal peptide (VEGFsp) with equal affinities (Herrera et al., 2005). The hypertension susceptibility in female F2(R × S) intercross rats was validated in humans, in a cohort from northern Sardinia (Glorioso et al., 2007). In the latter, the $\alpha_1$N,K-ATPase (*ATP1A1*) polymorphism was also tested and concordant with the rat data, and associated with *Dear* gene polymorphism, albeit in men (and not women). It is interesting that *ATP1A1* and *Dear* are coexpressed in both renal tubular cells and vascular endothelium: it strongly suggests a role in the regulation of blood pressure for these two genes. Altered *ATP1A1* and *Dear* functions in the endothelium could contribute, in combination, to endothelial dysfunction through a putative imbalance of endothelial repair to turnover, because *ATP1A1* is implicated in cell proliferation and *Dear* in angiogenesis. Likewise, *ATP1A1* and *Dear* in renal tubular epithelial cells could affect sodium homeostasis, because ET-1 decreases renal Na, K-ATPase activity. Based on this observation, a net decrease in ET-1/*Dear* activation could result in greater renal $Na^+$, $K^+$ ATPase activity and increased $Na^+$ reabsorption given the same sodium load, hence salt sensitivity. All these data come from one group of scientists, and the physiology and pharmacology of DEAR has not been studied in depth. We therefore do not know if ET-1 binding site is sensitive to the classical small molecule ET receptor antagonists.

Altogether, these data demonstrate that ET-1 effects are more complex than predicted so far: $ET_A$ and $ET_B$ receptor cooperation, heterodimerization, the newly discovered DEAR, and a possible bivalent ligand (Fig. 1) are possibilities that have not been fully explored. Based on our data in rat resistance pulmonary arteries (Sauvageau et al., 2006, 2007), we propose that $ET_A$ and $ET_B$ receptor heterodimerization is an important component in the pharmacological effects of ET-1, although no technique is yet available to evaluate the type of interactions that take place between the two receptors *in vivo.*

## B. Cardiovascular Effects of ET-1

In the systemic and pulmonary circulation, $ET_A$ receptors are expressed in the VSMC (Hosoda et al., 1991), while both $ET_A$ and $ET_B$ receptors are expressed on the surface of VSMC (Ogawa et al., 1991) and EC (Davenport et al., 1993). Both receptors in VSMC induce contraction and cell proliferation in the presence of ET-1 (Clozel et al., 1992; Docherty & MacLean, 1998; LaDouceur et al., 1993; MacLean et al., 1994; Shetty et al., 1993; Sumner et al., 1992). In EC, activation of $ET_B$ receptors activates the release of vasodilators and antiproliferative factors such as NO and $PGI_2$ (Clozel et al., 1992; de Nucci et al., 1988; Haynes & Webb, 1993; Muramatsu et al., 1999; Sato et al., 1995). The highest density of $ET_{A/B}$ receptors is found in the lungs and the heart (Simonson & Dunn, 1990).

ET-1 rapidly increases intracellular $Ca^{2+}$ via activation of the phospholipase C that hydrolyzes phosphatidyl inositol trisphosphate ($IP_3$) and the neutral diacylglycerol (DAG) (Resink et al., 1988). This is followed by a sustained phase of $Ca^{2+}$ influx associated with activation of secondary multiple intracellular events at the basis of ET-1-induced contraction, relaxation, and secretion (Fig. 1). The rise in $IP_3$ induces a fast and transient increase in $[Ca^{2+}]_i$ released from the reticulum, which is at the basis of the activation of membrane-bound channels leading to a sustained increase in $[Ca^{2+}]_i$ (Chen & Wagoner, 1991). This leads to numerous signals associated with $Ca^{2+}$-dependent pathways and $Ca^{2+}$/calmodulin-dependent pathways (Fig. 1): this includes activation of chloride channels (Haynes & Webb, 1993), the $Na^+/H^+$ exchanger resulting in cellular alkalinization and $Ca^{2+}$ influx through the $Na^+/Ca^{2+}$ exchanger (Grinstein & Rothstein, 1986; Koh et al., 1990), activates $Ca^{2+}$-induced $Ca^{2+}$ release from the reticulum via ryanodine receptors and $Ca^{2+}$-activated $K^+$ channels (Bialecki et al., 1989; Nelson et al., 1995; Simpson & Ashley, 1989). In addition, DAG activates PKC, which leads to numerous intracellular events (Fig. 1) including membrane translocation and activation of phosphokinases (Newton & Keranen, 1994), and damping of the $Ca^{2+}$ signal (Clerk et al., 1994).

Endothelin receptors are also expressed on the nuclear membrane of VSMC and cardiomyocytes, increasing nuclear $Ca^{2+}$ concentration, and endogenous nuclear protein kinase activities (Bkaily et al., 2000; Boivin et al., 2003).

**1. The Endothelium-Dependent Responses to ET-1—**Although ET-1 is known as a potent vasoconstrictor, in healthy animals, in which low levels of blood ET-1 are measured, intracoronary injections of low doses of ET-1 induce a decrease in vascular resistance (Fig. 2): in anesthetized dogs, for example, an intracoronary bolus injection of S6c, a selective $ET_B$ receptor agonist, induces a decrease in coronary resistance for doses lower than 1 $\mu$g (Teerlink et al., 1994). Likewise, the injection of ET-1 in isolated rat hearts leads to a drop in coronary perfusion pressure at low concentrations of ET-1 (Brunner et al., 2006). In coronary arterial rings isolated from young and healthy pigs, the activation of endothelial $ET_B$ receptors induces a significant relaxation (Climent et al., 2005) through the release of NO and $PGI_2$ (Callera et al., 2007; Rubanyi & Polokoff, 1994). In addition, we know that in the human forearm circulation, the increase in blood flow induced by $ET_A$ receptor blockade is blunted by $ET_B$ receptor antagonism and NOS inhibition (Verhaar et al., 1998), suggesting that endogenous ET-1 exerts a dilatory tone by stimulating endothelial $ET_B$ receptors.

Nonetheless, the dilatory role of $ET_B$ receptors is not significant in isolated human coronary arterial rings (Pierre & Davenport, 1998). However, such *ex vivo* studies are performed in human coronary vessels isolated from explanted hearts not of healthy subjects, but of patients undergoing cardiac transplantation for ischemic heart disease, vessels in which the expression of endothelial $ET_B$ receptors is limited, except in the neovascularization of the atherosclerotic plaque (Bacon et al., 1996), and with a pronounced endothelial dysfunction (Thorin, 2001). This suggests that in pathology the vasodilating effect of endothelial $ET_B$ receptor stimulation may be lost. Further data even suggest that in pathology, stimulation of endothelial $ET_B$ receptors might be detrimental by inducing effects such as cell adhesion or contraction (Bergdahl et al., 2001; Schneider et al., 2007; Sen et al., 2009).

One final argument supporting a dilatory effect of ET-1 on normal coronary arteries is that the basal production of ET-1 is five times greater toward the lumen than in the interstitial space (Brunner, 1995), which would favor $ET_B$ receptor stimulation on the endothelium, although other studies suggest, a polarized secretion of ET-1 toward the underlying VSMCs (Haynes & Webb, 1994; Unoki et al., 1999). Data from the study of Brunner demonstrated that the concentration of free ET-1 in the cardiac interstitial fluid never goes higher than 1 pg/ml (0.4 pM) in healthy animals, which is below the coronary constricting tone, supporting a vasodilatory tone associated with the stimulation of the endothelial $ET_B$ autoreceptors in the heart, in physiological conditions.

One should also not underestimate the importance of the concentration of ET-1 in determining the dilatory versus constricting coronary response, because of the heterogeneous distribution of ET-1 receptors as illustrated in cerebral versus pulmonary arteries (Sauvageau et al., 2009). Saturation experiments using iodinated ligands, competition experiments, and reactivity studies using ET-1 receptor antagonists and autoradiography revealed that the expression of $ET_A$ receptors is dominant compared to that of $ET_B$ receptors in the coronaries of explanted ischemic heart (Bacon & Davenport, 1996; Pierre & Davenport, 1998). The overall effects of ET-1 on vascular tone *in vivo* are the clear result of the balance between the contraction mediated by VSMC $ET_A$ and $ET_B$ receptors and the dilation mediated by endothelial $ET_B$ receptors (Callera et al., 2007; Thorin et al., 1999). This may explain that dual ET receptor antagonists such as bosentan or macitentan cause no vasodilation in healthy subjects but become vasodilators in pathological vascular beds.

**2. Smooth Muscle Contraction, Inflammation, and Vascular Diseases—**In rats, injection of bosentan, a dual $ET_{A/B}$ receptor antagonist, does not reduce blood pressure; after blockade of NO production, however, bosentan reduces blood pressure (Richard et al., 1995). This therefore suggests that NO inhibits ET-1-dependent activity *in vivo*. Bosentan and BQ123 dilate isolated and pressurized rabbit mesenteric arteries in no-flow conditions whether or not NO synthase is blocked (Nguyen et al., 1999). In these latter conditions, however, one might expect a lower influence of NO on the regulation of the vascular diameter since shear stress is nil, therefore favoring the effects of endogenous constrictors such as ET-1. In rat isolated pulmonary arteries, the contraction induced by ET-1 is both $ET_A$ and $ET_B$ receptor dependent, while in rat cerebral arteries, it is mostly $ET_A$ receptor mediated, in agreement with the receptor expression profile in these arteries (Sauvageau et

al., 2007, 2009). Coronary vessels, because of the ET-1/NO interdependence and due to their unique hemodynamic features (see later), are very susceptible to higher circulating and locally produced ET-1. The coronary endothelium is prone to dysfunction and highly sensitive to damage, which, with time, accumulates faster in the coronary than in other vascular beds. In addition, coronary endothelial dysfunction is associated with a decline in the contribution of NO in favor of a growing influence of ET-1 (Alonso & Radomski, 2003). Is the increased influence of ET-1 with time in the coronary bed only secondary to the loss of NO or is it due to a change in the ratio of $ET_A$ and $ET_B$ receptor expression? In any case, this supports the proinflammatory and proconstricting role of ET-1 via its predominant activation on smooth muscle receptors (Griendling et al., 2000; Marsden & Brenner, 1992; Sprague & Khalil, 2009), and could become the basis for the use of ET receptor antagonists to treat coronary artery diseases (CAD).

In cultured cells, it has been shown that ET-1 mRNA is upregulated by inflammatory factors such as TGF-β, TNF-α, interleukins, insulin, and ANG II, and downregulated by NO, $PGI_2$, and shear stress (Boulanger & Luscher, 1990; Brunner et al., 1995; Kohno et al., 1992; Kourembanas et al., 1993; Maemura et al., 1992; Prins et al., 1994). When considering these regulatory mechanisms within the coronary circulation, shear stress is a key element and NO is the key effector (Liu & Gutterman, 2009). In contrast to other vascular beds, wall shear stress in coronary arteries is uneven during the cardiac cycle (Heusch, 2008) and mechanical stress is therefore greatest in the coronary circulation (Thorin & Thorin-Trescases, 2009). In turn, it is not surprising that the coronary circulation is the prime site for endothelial dysfunction. It has been reported that because of these unique physiological hemodynamic features, coronary arteries display an unusual gene pattern when compared to the aorta: a fivefold lower eNOS and a 2.5-fold higher ET-1 mRNA expression (Dancu & Tarbell, 2007). Such a pattern predisposes coronary arteries to endothelial dysfunction and atherosclerosis. Therefore, based only on its physiological characteristics, the coronary circulation should be prone to an increased influence of ET-1 with age: the accumulation of age-related damages would favor ET-1 expression in contrast to that of eNOS and exacerbate endothelial dysfunction (Fig. 1).

Patients with atherosclerosis have elevated plasma levels of ET-1, and an upregulation of ET-1 and its receptors has been described in atherosclerotic arteries and plaques (Barton & Yanagisawa, 2008; Dagassan et al., 1996; Fan et al., 2000; Lerman et al., 1991). BigET-1 and ET-1 immunoreactivity has been found in atherosclerotic regions (Dashwood et al., 1998b; Hasdai et al., 1997). These observations have led to the hypothesis that ET-1 may be associated with the pathogenesis of atherosclerosis (Dashwood & Tsui, 2002; Ivey et al., 2008). In 1998, an important preclinical study (Barton et al., 1998) demonstrated that chronic $ET_A$ receptor inhibition improved aortic endothelial dysfunction and reduced the development of atherosclerosis in ApoE knockout mice. Several studies have subsequently demonstrated the beneficial effects of acute intracoronary infusion of the $ET_A$ receptor antagonist BQ123 on coronary diameter and coronary flow in patients with CAD. When narrowing the analysis of the dilatory effects of BQ123 to angiographically normal vessels, vessels with plaques and at stenosis, a higher dilation was observed after intracoronary infusion of BQ123 (40 nmol/min, for 60 min) in patients with CAD (Kinlay et al., 2001); in this study, compared with the dilation to nitroglycerin, ET-1 contributed to 39% of coronary

vasomotor tone in healthy and angiographically clean vessels, 74% of tone in CAD arteries, and 106% of tone at stenosis. The contribution of NO was, however, not determined: one would assume that the more severe the disease condition, the less NO would be produced, and the more ET-1 would contribute to tone. This hypothesis was tested in 44 patients with CAD in a study published that same year: Halcox et al. provided the first evidence that ET-1, via $ET_A$ receptors, contributed to the reduction of endothelial dilatory function (Halcox et al., 2001). The greatest improvement associated with the intracoronary infusion of the $ET_A$ receptor antagonist was observed in patients with the greatest endothelial dysfunction as determined in the presence of acetylcholine (Halcox et al., 2001), suggesting that ET-1 contributes to the acute inactivation of NO. However, the tachyphylaxis of NO-dependent dilation occurring following systemic injections of ET-1 could also explain the apparent inactivation of NO by ET-1 (Le Monnier de Gouville et al., 1990); alternatively, we observed that chronic infusion of LU-135252 increased VSMC-sensitivity to NO, suggesting that ET-1 may regulate negatively the sensitivity of the soluble guanylate cyclase (Thorin et al., 2000). Both the selective $ET_A$ receptor (BQ123) antagonist and the combination of selective $ET_A$ (BQ123) and $ET_B$ receptor (BQ788) antagonists improved endothelium-dependent dilation in the coronary arteries of patients with CAD (Bohm et al., 2008). In agreement with these data, using isolated human coronary arteries from idiopathic and atherosclerotic cardiomyopathic hearts, we demonstrated that ET-1-dependent constrictions became more pronounced when the endothelial function was altered (Thorin et al., 1999). Recently, a work by Dr Lerman's group (Reriani et al., 2010) showed that a chronic (6 months) treatment of patients with premature atherosclerosis with the ETA receptor antagonist atrasentan (10 mg/day) improves coronary endothelial function. Taken together, these data strongly suggest that ET-1 contributes to inactivate the dilatory function of the endothelium in the coronary arterial bed. Hence, the functional contribution of ET-1 is precocious and appears to rise with the severity of CAD.

ET-1, at low concentrations, potentiates coronary contractile responses to other vasoconstrictor substances such as norepinephrine and serotonin (Garcia-Villalon et al., 2008; Rubanyi & Polokoff, 1994; Thorin et al., 1998). In human cerebral arteries, a reduction in endothelium-derived ET-1 accounts for the dilatory effects of endothelial $\alpha_2$-adrenergic receptor stimulation (Thorin et al., 1998). Consequently, even subthreshold concentrations of ET-1 may regulate vascular tone and reactivity in conditions where NO production is reduced, that is, with age and in patients presenting with risk factors for cardiovascular diseases.

The potential physiological roles of $ET_B$ receptors, in addition to acting as clearance receptors for ET-1 and stimulating NO release, remain poorly understood. This is likely due to the limited final effects of the stimulation of endothelial $ET_B$ receptors on the *in vitro* vascular function and the possible change in the expression of ET-1 receptors during the development of pathologies as evidenced in pulmonary hypertension (Sauvageau et al., 2009). A change in receptor expression is likely to change the pharmacology of the system. The consequences of receptor inhibition in young and healthy subjects or in old and diseased patients should obviously be different. Since most clinical data have been collected in an elderly population most likely showing some degree of endothelial dysfunction, it is quite possible that our understanding of ET-1 function as a proconstrictor and proinflammatory

factor is only a reflection of these data and experimental environment, and thus may not illustrate the effects of ET-1 in young and healthy subjects. In support of this statement, the induction of endothelial damage eliminates $ET_B$-receptor-dependent relaxation in pig coronary arteries (Climent et al., 2005). The seminal demonstration that acetylcholine induces a contraction of coronary arteries in patients with CAD, but a dilation otherwise (Ludmer et al., 1986), is a good example of such a case.

Therefore, based on the literature reviewed so far, one can infer that at physiological and low concentrations, ET-1 predominantly induces dilations, while at pharmacological concentrations it induces contractions. The impact of the inevitable endothelial dysfunction when using isolated arteries from explanted human hearts may have led researchers to underestimate the endothelial dilatory component of ET-1. The production of NO may be reduced, but an alternative explanation may be the loss of coupling between the $ET_B$ receptor and the NO pathway without affecting the ability of NO to clear ET-1 from the circulation. For example, acetylcholine induces a contraction of coronary vessels isolated from patients with ischemic heart disease, but substance-P still produces near-maximal relaxation by stimulating NO production (Thorin, 2001). A change in the expression or coupling of the endothelial $ET_B$ receptor cannot be excluded in an elderly population (> 65 years of age) and in patients with CAD.

**3. Pulmonary Circulation—**The pulmonary circulation is highly susceptible to elevated levels of ET-1 which have been associated with PAH (Stewart et al., 1991). Circulating levels of ET-1 are a good marker of disease severity (pulmonary vascular resistance, right atrial pressure, and pulmonary artery oxygen saturation) and predict poor prognosis (Stewart et al., 1991). Upregulation of ET-1 production by the lungs and changes in ET receptor expression could be at the basis of the dysregulation and PAH (Sauvageau et al., 2009; Takahashi et al., 2001). In animal models of PAH, both dual antagonists of $ET_A$ and $ET_B$ receptors (bosentan) and selective $ET_A$ receptor antagonists (sitaxsentan, atrasentan, TBC-3711) are effective in reducing pulmonary artery resistance and inhibiting vascular remodeling. In humans, both types of antagonists are used (Kirkby et al., 2008; Motte et al., 2006).

Bosentan (Tracleer) was approved for the treatment of PAH in 2001 based on two clinical trials, “Study 351” with 32 class III patients with idiopathic PAH or associated with systemic sclerosis (Channick et al., 2001) and the important BREATHE-1 study that included 150 patients with idiopathic PAH, 47 with systemic sclerosis–associated PAH, and 16 with systemic lupus erythematosus–associated PAH (Rubin et al., 2002). In the latter study, bosentan improved exercise capacity, the functional class, and increased the time to clinical worsening. Sitaxsentan was approved for treatment of PAH in 2006 based on the STRIDE-1 results (Barst et al., 2004). Recently, ambrisentan has been approved for the treatment of PAH (Galie et al., 2008). No clinical advantages have been demonstrated between dual ET receptor antagonist and selective $ET_A$ receptor antagonists.

**4. Cardiac Myocyte Function and Heart Failure—**ET-1 has positive cardiac inotropic effects in healthy animals and humans (Kang & Walker, 2006; Katoh et al., 1998; Kelly et al., 1990; Li et al., 1991; Pieske et al., 1999), but not in failing human hearts (MacCarthy et

al., 2000; Pieske et al., 1999). ET-1 enhances myocyte contractility by activating $ET_A$ receptor-phospholipase Cβ-PKCε signaling complexes preferentially localized in cardiac T-tubules (Robu et al., 2003). It has been shown that ET-1 is devoid of any significant effects on basal L-type $Ca^{2+}$ channel activity, but exerts a potent inhibitory effect against isoprenaline-enhanced L-type $Ca^{2+}$ channel current (He et al., 2000; Watanabe & Endoh, 2000). This effect is mediated through $ET_A$ receptors coupled to pertussis toxin–sensitive G proteins (He et al., 2000; Thomas et al., 1997).

As mentioned earlier, ET-1 has growth-promoting effects (Inada et al., 1999). Because there is a correlation between ventricular mass and ET-1 concentration in the blood, it is possible that ET-1 contributes to the ventricular hypertrophy in patients with ischemic heart failure and dilated cardiomyopathy (Tsutamoto et al., 2000) and in rats following coronary artery ligation (Loennechen et al., 2001). ET-1 also promotes sympathetic tone, especially in heart failure as demonstrated in rabbits (Liu et al., 2001) and dogs (McConnell et al., 2000). This may partly explain the proarrhythmic effects of ET-1 (Burrell et al., 2000; Yorikane & Koike, 1990; Yorikane et al., 1990), while circulating levels of ET-1 have been associated with arrhythmia in patients with decompensated heart failure (Aronson & Burger, 2003a, 2003b; Aronson et al., 2001).

**5. Renal Effects of ET-1—**The effects of ET-1 on the kidney are complex. Exogenous administration of ET-1 induces a vasoconstriction in the renal cortex and a vasodilatation in the medulla (Rubinstein et al., 1995). The latter is mediated by $ET_B$ receptors while the former is dependent on both $ET_A$ and $ET_B$ receptor activation (Dhaun et al., 2006). Acutely, selective $ET_A$ receptor antagonism with BQ123 reduced blood pressure, proteinuria, and pulse wave velocity on top of standard treatment in patients with nondiabetic chronic kidney diseases (Dhaun et al., 2009). In diabetic patients with chronic kidney disease, however, chronic $ET_A$ receptor antagonism with avosentan is deleterious due to fluid overload and congestive heart failure (Mann et al., 2010). In the inner-medullary-collecting duct of mice, ET-1 induces an autocrine natriuretic and diuretic effect which seems mediated by $ET_B$ receptors, since specific inner-medullary-collecting duct deletion of $ET_B$ but not $ET_A$ receptors leads to salt-sensitive hypertension (Bagnall et al., 2006; Ge et al., 2006). However, the renal effects of $ET_B$ receptor antagonism or deletion are inhibited by $ET_A$ receptor antagonism, showing that it is the reactive increase in ET-1 acting on $ET_A$ receptors, not the deletion of $ET_B$ receptors *per se*, which is responsible for hypertension and tissue injury (Matsumura et al., 2000). Dual ET antagonists seem to give very low rates of fluid retention and edema in the clinical setting. In PAH clinical trials with Tracleer, combined adverse events of fluid retention or edema were reported in 1.7% (placebo-corrected) of patients (Tracleer US package insert, 2009). In a Phase II study in hypertensive patients, the novel dual ET antagonist macitentan did not cause peripheral edema (Press release Actelion Dec 2006). It is therefore possible that ET receptor antagonists can be used safely in patients with renal diseases, but this remains to be validated in a proper clinical trial.

## III. Conclusion

Numerous clinical developments are ongoing with ET receptor antagonists (Aubert & Juillerat-Jeanneret, 2009). Our understanding of receptor pharmacology in general is changing with the appearance of new concepts including dimerization and G-protein-independent signaling. These changes apply to ET-1 and its receptors. One major weakness, however, which applies to many other pharmacological systems, is our lack of knowledge of the evolution of ET-1 and its receptors in the aging human and how this influences cardiovascular function in combination with risk factors for cardiovascular diseases. The critical role for ET-1 in controlling cardiovascular function is evident by the fact that its clinical importance was established within a few years after its discovery. It is likely that work in this area will continue to yield novel therapies for the treatment of cardiovascular disease.

## Acknowledgments

The laboratory of Dr. Eric Thorin is supported by the Canadian Institutes for Health Research (MOP 14496 and 89733), the Heart and Stroke Foundation of Quebec, and the Foundation of the Montreal Heart Institute.

## Abbreviations

| | |
|---|---|
| **7-TM** | 7-transmembrane domain |
| **ANG II** | angiotensin II |
| **EDCF** | endothelium-derived constricting factor |
| **ET-1** | endothelin-1 |
| **GPCR** | G protein-coupled receptor |
| **PAH** | pulmonary arterial hypertension |
| **$PGI_2$** | prostacyclin |
| **S6c** | sarafotoxin 6c |

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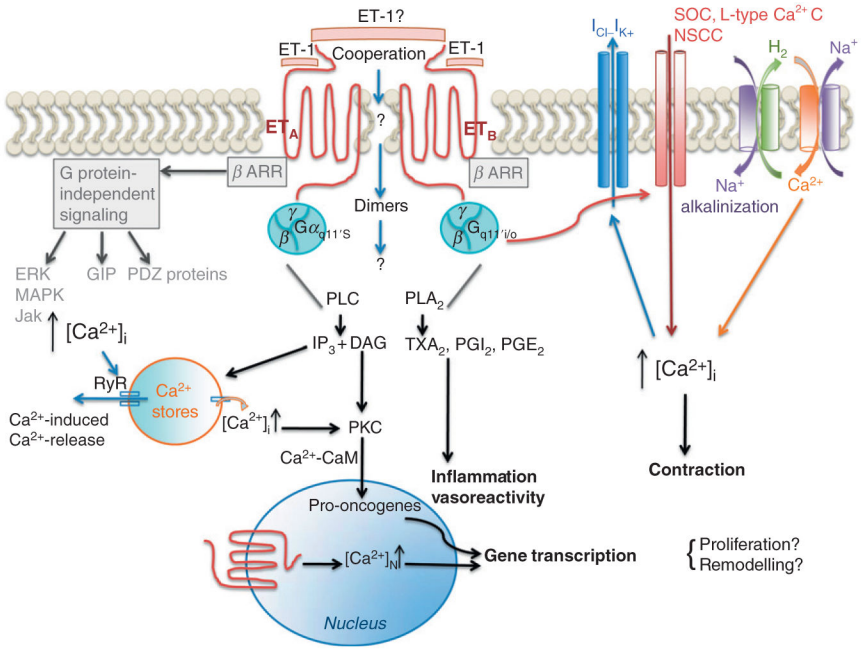


**FIGURE 1. The pleiotropic nature of ET-1 receptor signaling**

Schematic representation of the multiple pathways activated by 7-transmembrane $ET_A$ and $ET_B$ receptors either directly dependent on G protein activation or independent of G protein activation such as through direct interaction with β-arrestin or PDZ-domain-containing proteins that can act as scaffolds. In addition, ET-1 may act as a bivalent ligand leading to both $ET_A$ and $ET_B$ receptor activation and possibly dimerization, although this remains to be demonstrated. The signaling pathways activated by either a bivalent ET-1 or a dimerization remain unexplored. $G\alpha_{s/i/o/q/11}$, heterotrimeric G protein α subunits of different classes; β arr, β-arrestin; PKC, protein kinase C; Jak, Janus kinase; GIP, other GPCR interacting proteins; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase.

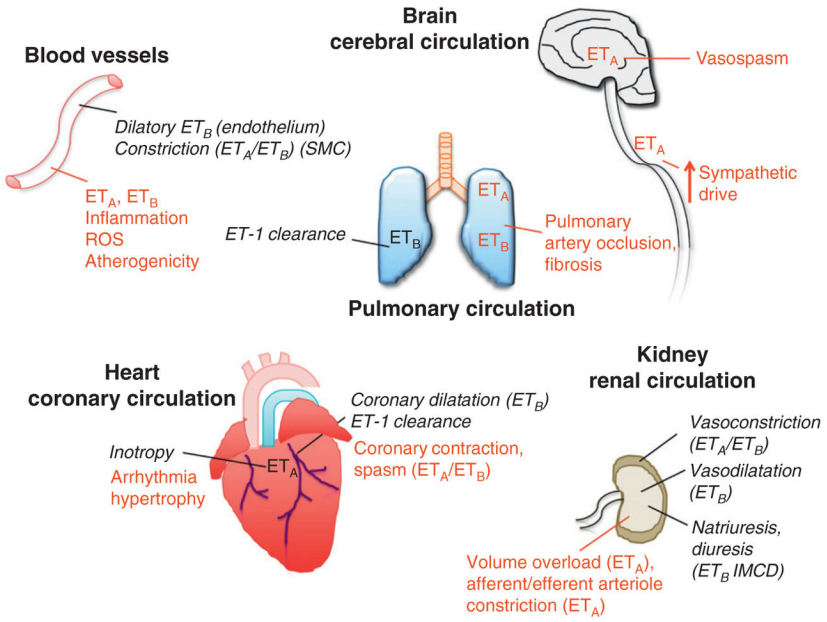


**FIGURE 2. Multiple effects of ET-1 in the cardiovascular system**

Physiological responses are presented in italic and black while the responses associated to pathological conditions are in light grey (and in red in the online version).