**Endothelin—role in vascular disease**

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It is now two decades since it was demonstrated that ET-1 is one of the most powerful vasoconstrictors in biology. ET-1 mediates its effects through two membrane G-protein coupled receptors, ET\(_A\) and ET\(_B\), which exhibit a wide tissue distribution including the endothelial cells, vascular smooth muscle cells and adventitial fibroblasts. In recent years, ET-1 has been identified as a key player of endothelial dysfunction in various cardiovascular, autoimmune and CTDs. Endothelial dysfunction results from endothelial cell injury subsequently leading to the generation of an inflammatory process and endothelial cell activation. Thus, beyond its known ‘classical’ vasoactive effects, ET-1 is additionally considered to be an important mediator in vessel remodelling ultimately leading to major changes in cellular and tissue architecture; it also appears to function in conjunction with other growth factors and cytokines. Consequently, ET-1 receptor antagonists may be useful in ameliorating progression of vascular dysfunction and vascular disease due to their ability to negatively modulate vasoconstrictor pathways, cytokines and inflammatory markers production, and growth factor effects. This review briefly summarizes the current knowledge on the role of ETs in vascular dysfunction and vascular disease, with a particular emphasis on ET-1 in CTDs.

**Key words:** Endothelin A/B receptors, Endothelin receptor antagonists, Endothelium, Vascular pathology, Tissue remodelling, Fibrosis, Signal transduction, Fibroblasts, Vascular smooth muscle cells.

**Introduction**

The ET family comprises three vasoactive peptides (ET-1, ET-2 and ET-3). A fourth isoform (ET-4) has been reported in the rat and mouse as the analogue to human ET-2 \([1]\). ETs are processed from inactive precursor pro-polypeptides by a subgroup of membrane-bound zinc metalloproteases, the ET-converting enzymes (ECEs). The three ET isoforms bind to two cell surface receptors: ET receptor subtype A (ET\(_{A\, R}\)) and subtype B (ET\(_{B\, R}\)). ET\(_{C\, R}\), a third receptor subtype, has been cloned from *Xenopus laevis* oocytes, but molecular biology studies failed to identify a homologue in mammalian tissues. ET-1 has the highest affinity for ET\(_{A\, R}\), followed by ET-2 and ET-3, with all ETs exhibiting equal affinity for ET\(_{B\, R}\) \([2]\). There is an ongoing debate on the exact roles of ET\(_{A\, R}\) and ET\(_{B\, R}\) receptors, but important functions include eliciting vasoconstriction and vasodilatation [via release of nitric oxide (NO)], ET clearance, salt balance, cell proliferation and extracellular matrix (ECM) production. A general feature of ET receptors is that they are abnormally expressed in various diseases associated with vasoconstriction, vasospasm and vascular hyper-trophy \([3]\). ET-1 represents the major isoform in humans (mostly referred to as ET in the literature) and has been the most studied. The ETs have significant roles in controlling vascular tone by acting on vascular smooth muscle cells and it has been suggested that ETs are involved in the pathophysiology of various vascular diseases \([1, 4, 5]\). This review briefly summarizes the current knowledge on the role of ETs in vascular dysfunction and vascular disease, with a particular emphasis on ET-1.

**Endothelial function and dysfunction**

The vascular endothelium is the gatekeeper of vascular function, significantly contributing to cardiovascular homeostasis by releasing mediators able to modulate vascular tone, haemostasis, recruitment and activity of inflammatory cells, vascular permeability and fluid balance; it also regulates angiogenesis. Endogenous vasomotor tone is controlled via the production of vasodilatory and vasoconstrictive molecules. The main endothelium-derived relaxing factors are NO and prostacyclin while ET-1 represents the most potent vasoconstrictor. An intact balance of vasoconstrictive and vasodilatory molecules not only provides a normal vascular tone but also serves to inhibit ECM deposition and to prevent smooth muscle cell proliferation. A fully functioning endothelium is particularly critical during times of tissue growth and repair.

Endothelial dysfunction is defined by an impaired vascular reactivity, but it also refers to a pre-inflammatory and pro-thrombotic state \([5]\). Endothelial dysfunction has been described in many cardiovascular and metabolic disorders such as arterial hypertension, pulmonary hypertension, coronary heart disease, dyslipidaemia, peripheral vascular disease and type I and II diabetes; it also plays a role in diseases characterized by inflammation, excessive scarring and fibrosis (e.g. *fibrotic* CTDs) \([3, 5]\). It is perceived that endothelial dysfunction results from endothelial cell injury subsequently leading to the generation of an inflammatory process and endothelial cell activation. Endothelial cell injury may be triggered by various mechanisms such as bacterial or viral infection, abnormal regulation of reactive oxygen species, hypoxia, turbulent blood flow and shear stress or environmental irritants \([6]\). In autoimmune diseases, the presence of anti-endothelial cell antibodies may also contribute to endothelial cell injury and apoptosis.

The initial response to endothelial injury involves cell signalling and calcium metabolism by changing concentrations of NO, prostaglandins, ET-1, von Willebrand factor and tissue plasminogen activator. Hence, this early response is rapid (within minutes) and transient, and is important for cell contraction. A second, phenotypic response is slower and induces changes in cell surface characteristics and alters the underlying basement membrane and the smooth muscle cells that surround the endothelium. These changes are triggered by the activation and proliferation of vascular smooth muscle cells (associated with intermediate and large vessels as well as muscular arteries), pericytes (associated with microvessels and capillaries) and other cells of the blood vessel wall. Growth factors involved in the deposition of ECM also play a role. The second response occurs within hours and days, involves transcriptional activation and stimulation of modifying genes that control matrix, potentiates the contractile system and alters tissue composition. Eventually, this leads to vessel remodelling and major changes in cellular and tissue architecture \([7]\).
Endothelin-1 and endothelial dysfunction

A large body of evidence suggests that ET-1 is a key player of endothelial dysfunction in cardiovascular diseases ranging from coronary artery disease, peripheral artery disease and stroke to hypertension and varicose veins. Much evidence for a pathological role of ET-1 in these conditions is based on elevated blood or tissue ET-1 levels that have been demonstrated in both animal models and studies on patients [1, 4, 5, 8, 9]. Endothelial dysfunction appears to precede the clinical manifestations of many of these cardiovascular disorders, hypertension for example, and also atherothrombosis, where abnormal vasoconstriction can be observed at the future site of plaque development [10].

Endothelial dysfunction with involvement of ET-1 has also been described in autoimmune diseases such as scleroderma, lupus and MCTD [5]. Although the most extensively studied action of ET-1 is the regulation of the basal constrictor tone of blood vessels, it elicits a number of cell-type specific responses. For example, ET-1 up-regulates many genes in inflammatory lesions and in the tissues affected by cardiovascular diseases. In smooth muscle cells and fibroblasts, it modulates the expression of ECM and MMPs that may lead to promotion of tissue remodelling and fibrosis [11]. The latter is most likely triggered by elevated production of TGF-β and PDGF, both are key profibrotic factors [12]. In fibroblasts and endothelial cells, ET-1 increases adhesion molecule expression that facilitates leukocyte-fibroblast interactions. Furthermore, ET-1 modulates inflammatory responses in macrophages such as nuclear factor-κB (NF-κB) activation, release of free radicals and increased levels of IL-8, monocyte chemoattractant protein-1 and TGF-β [3].

Recently, it has been suggested that decreased NO levels, induced by ET-1, may contribute to endothelial dysfunction [5]. Indeed, it was shown that endothelium-restricted overexpression of ET-1 caused endothelial dysfunction and a decrease in NO [13]. This is supported by pre-clinical and clinical data suggesting that selective ET receptor antagonists improve NO bioavailability and endothelial function in pathological situations [5].

Interaction between ET-1 and receptors

ET-1 is produced by endothelial and mesenchymal cell types, such as fibroblasts and smooth muscle cells; it acts in an autocrine and paracrine manner by binding to ET_A and ET_B receptors. These receptors have seven transmembrane domains and are coupled to G proteins. ET-1 is produced as a pre-pro transcript that is translated into a pre-pro polypeptide of 212 amino acids. Endothelial cell stimulation promotes the synthesis of pre-pro-ET-1 polypeptides. The pre-pro polypeptide is then processed by a series of converting enzymes to pro-ET-1 (or big ET-1), which is a large, relatively inactive ET molecule exhibiting 1% of the activity of the fully functional 21-amino acid peptide. Big ET-1 is then converted by the membrane-bound enzyme ET converting enzyme-1 into the functional, mature 21-amino acid form. Subsequently, this mature ET-1 is released locally and interacts with specific ET receptors expressed on the surface of vascular smooth muscle cells and adventitial fibroblasts (ET_A and ET_B), and with receptors located on endothelial cells (ET_B) [3]. Recognition of ET-1 by these receptors activates intracellular signalling pathways and cascades that result in rapid alterations in cell activity and function, and initiates transcriptional programmes.

ET receptors exhibit a broad tissue distribution and are expressed in kidney, liver, lung and skin suggesting that they have important and wide-ranging biological activities in vivo. In the context of vascular dysfunction and vascular disease, it is thought that potential pharmacological interventions should target the interaction between ET-1 and endothelial cells, smooth muscle cells and fibroblasts. In fact, single or dual ET receptor antagonists are available that block the effects of ET-1 on such target cells. Other potential approaches for intervention include the selective blockade of ET-converting enzyme-1 to prevent production of the active peptide from big ET-1, the disruption of the intracellular signalling processes that are induced by ETA/ETB receptor binding, and the prevention of transcriptional activation triggered by the interaction of ET-1 with its receptors. For the latter, basic science studies have been successful in inhibiting the effects of ET-1 effectively [3].

In summary, ETs play a major role in vascular dysfunction and vascular disease. Beyond its known vasoconstrictive effects, ET-1 is an important mediator in vessel remodelling, which ultimately results in major changes in cellular and tissue architecture.

### Rheumatology key messages

- ETs are rapidly produced by endothelial cells in response to tissue injury; they play a major role in vascular dysfunction and vascular disease.
- ET-1 represents the most common ET in humans: it mediates its effects through the two membrane receptors, ETA and ETB, which exhibit a broad tissue distribution including the endothelial cells, vascular smooth muscle cells and adventitial fibroblasts.
- Endothelial dysfunction has been described in many cardiovascular and metabolic disorders, and in diseases characterized by inflammation and fibrosis (e.g. fibrotic CTDs).
- Beyond its known vasoconstrictive effects, ET-1 is an important mediator in vessel remodelling by working in conjunction with various growth factors and cytokines.
- ET-1 receptor antagonists may be useful in ameliorating progression of vascular dysfunction and vascular disease due to their ability to negatively modulate vasoconstrictor pathways, cytokines and inflammatory markers production and growth factor effects.

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