

# ENDOTHELIN ANTAGONISM IN PULMONARY HYPERTENSION, HEART FAILURE, AND BEYOND

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The discovery in 1988 of endothelin-1 (ET-1) by Yanagisawa and colleagues in Japan represented a landmark in the field of cardiovascular research.<sup>1</sup> A combination of molecular and pharmacological approaches revealed ET-1 as the most powerful vasoconstrictor yet identified in biological systems. Since its discovery, a great deal of effort has been made towards gaining a better understanding of the key roles—developmental, physiological, and pathological—played by this peptide, particularly with regard to the cardiovascular system. In this review, we will describe current knowledge on the endothelin system and focus on the cardiovascular effects of ET-1 and its antagonism. We will review the evidence for the two therapeutic areas mainly investigated, pulmonary arterial hypertension and heart failure, as well as outline its important role in hypertension, renal dysfunction, and atherosclerosis.

## MOLECULAR BIOLOGY OF ENDOTHELIN-1

### Endothelin synthesis and clearance

The endothelin family consists of three closely related peptides, ET-1, ET-2, and ET-3, each 21 amino acids in length and derived from separate genes. ET-1<sub>[1–21]</sub> is the main isoform produced in the cardiovascular system and about which most is known; ET-2 is mainly produced within the kidney and intestine, whereas ET-3 is predominantly found within the central nervous system (tables 1 and 2). However, the roles of ET-2 and ET-3, except in embryonic development, remain unclear.

ET-1 is not stored and released but instead generated in response to a range of stimuli, which vary between different tissues (fig 1).<sup>2</sup> It is generated mainly in endothelial cells of blood vessels and requires several processing steps before the mature peptide is formed (fig 1). The final step in the ET-1 pathway involves cleavage of the 38 amino acid “big ET-1” by the highly selective membrane bound metalloproteinase, endothelin converting enzyme (ECE-1). ECE independent pathways of ET-1 formation have also been described: they involve tissue chymases cleaving big ET-1 to produce alternative intermediary peptides, such as ET-1<sub>[1–31]</sub>, which has recently been shown to cause vasoconstriction in the human skin microcirculation.<sup>w1</sup>

ET-1 is predominantly secreted abluminally and, as such, exerts paracrine actions. Therefore, interpretation of ET-1 and big ET-1 plasma concentrations require some caution. Indeed, effects on ET clearance rather than production are a major determinant of plasma concentration. Nevertheless, they may be useful as an index of ET-1 synthetic activity.

Although its biological effects may last considerably longer, the plasma half life of ET-1 is < 2 minutes, owing to its efficient extraction in the pulmonary and renal vascular beds. This extraction involves binding to cell surface clearance ET<sub>B</sub> receptors, followed by internalisation and degradation, probably within lysosomes. Endothelins are also degraded by neutral endopeptidases (NEP), which are mainly found in the brush border vesicles of the proximal tubules of the kidney.

### Endothelin receptors

ET-1 exerts its actions through binding to specific receptors, the so called type A (ET<sub>A</sub>) and type B (ET<sub>B</sub>) receptors. Both of them are G protein coupled transmembrane proteins, with different molecular and pharmacological characteristics and functions based on their location. ET-1 binding to these receptors results in activation of the phosphatidyl inositol phospholipase C pathway and initiates an array of intracellular events, with both short and long term effects, such as rapid increase in intracellular calcium levels, activation of protein kinase C, and nuclear signalling mechanisms.

The ET<sub>A</sub> receptor is predominantly expressed in the vascular smooth muscle cells and cardiac myocytes. Its interaction with ET-1 results in vasoconstriction and cell proliferation. In contrast, the ET<sub>B</sub> receptor is predominantly expressed on vascular endothelial cells and is linked to an inhibitory G protein. Its activation results in nitric oxide (NO) induced vasodilatation and prostacyclin release. This receptor is also present, at a much lower level, on the vascular smooth

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**Table 1** Sites of production of ET-1, ET-2, and ET-3

ET-1	ET-2	ET-3
▶ Endothelial cells	▶ Kidney epithelial cells	▶ Neurons
▶ Vascular smooth muscle cells	▶ Gastrointestinal stromal cells	▶ Glia
▶ Epithelial cells		▶ Adrenal cells
▶ Hepatocytes		▶ Lung epithelial cells
▶ Neurons		▶ Gastrointestinal stromal cells
▶ Astrocytes		▶ Kidney epithelial cells

muscle cells, where its activation can contribute to vasoconstriction. In addition, ET<sub>B</sub> receptors play a determinant role in the clearance of circulating ET-1, particularly in the pulmonary and renal vascular beds. In the absence of disease, ET-1 actions result in a complex modulation of vasomotor tone, tissue differentiation, and cell proliferation, driven by the interplay between effects on ET<sub>A</sub> and ET<sub>B</sub> receptors. However, experimental evidence suggests that, in pathological states, ET receptors are differently regulated, thus contributing to unbalanced effects tending towards vasoconstriction and cell proliferation.

The more detailed basic science and molecular biology of the endothelins have been reviewed elsewhere.<sup>2-4 w2</sup>

### PHYSIOLOGICAL ACTIONS OF ENDOTHELIN-1

Understanding the role of ET-1 in human physiology has followed directly from the development of a series of endothelin agonists and, most importantly, antagonists.

#### Endothelin agonism

As yet, a selective ET<sub>A</sub> receptor agonist has not been discovered. With regard to ET<sub>B</sub> receptors, sarafotoxin-6c and ET-3 have been used as agonists, given their relative selectivity for the ET<sub>B</sub> over the ET<sub>A</sub> receptors. In man, ET-1 produces a slow onset dose dependent vasoconstriction that is sustained for up to two hours.<sup>w3</sup> This response is largely abolished by co-infusion of an ET<sub>A</sub> receptor antagonist, suggesting that ET-1 vasoconstriction in resistance vessels is predominantly ET<sub>A</sub> mediated.<sup>5</sup> This effect is preceded by a transient vasodilatation that seems to be mediated by endothelial ET<sub>B</sub> receptors.

Systemic intravenous infusion of ET-1 and big ET-1 causes a dose dependent pressor effect, as well as a reduction in heart rate.<sup>w4</sup> These systemic effects are associated with falls in coronary blood flow and coronary sinus oxygen saturation, indicating the potential role of ET-1 in the regulation of coronary vascular tone.

**Table 2** Classification of endothelin receptors

	ET <sub>A</sub>	ET <sub>B</sub>
▶ Agonist potency	ET-1 > ET-2 >> ET-3	ET-1 = ET-2 = ET-3
▶ Selective agonist	None	Endothelin-3, sarafotoxin-6c, BQ3020, IRL1620
▶ Main actions	Vasoconstriction Mitogenesis Angiogenesis Matrix formation	Vasodilatation Vasoconstriction Natriuresis ET clearance

#### Endothelin antagonism

Various preclinical and clinical data support a pathogenic role for ET-1 in a number of disease states. This has fuelled research into the development of endothelin antagonists, in search of the potential benefit that might derive from their use in clinical practice. They are classified into two major subgroups: ECE inhibitors and endothelin receptor antagonists.

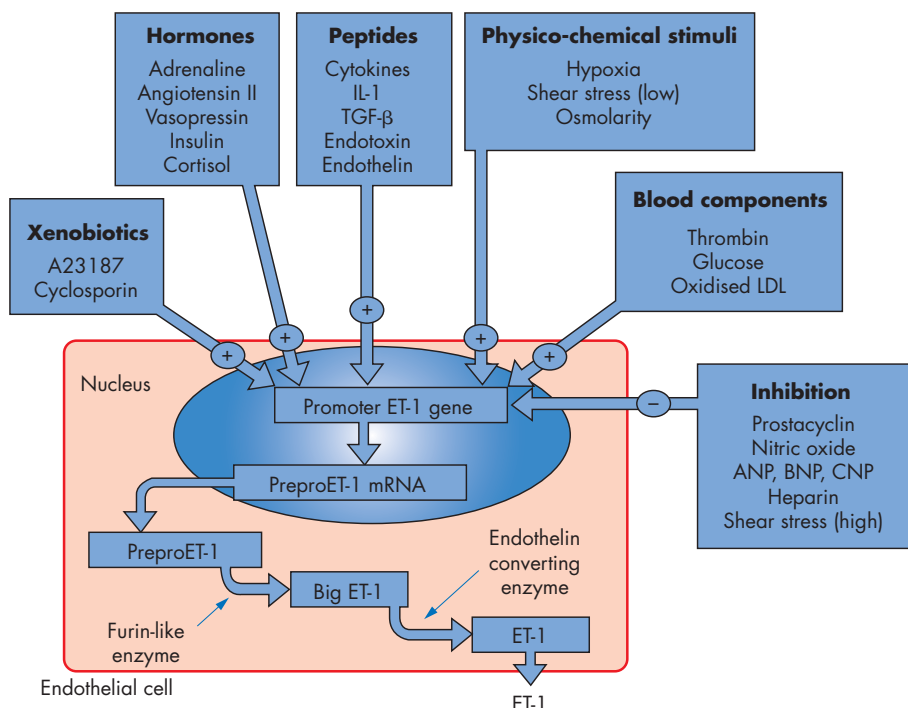
#### ECE inhibition

Inhibition of ECE blocks the conversion of big ET-1 to ET-1 and is associated with vasodilatation and hypotension.<sup>5</sup> Most ECE inhibitors currently under development also inhibit neutral endopeptidase (NEP), so that they have the combined effect of inhibiting ET-1 production and potentiating endogenous vasodilator mediators metabolised by NEP, such as atrial natriuretic peptide and bradykinin (table 3). This inhibition might also be extended to include triple inhibition of ECE, NEP, and angiotensin converting enzyme (ACE), with a potentially beneficial action in pathological conditions such as heart failure and renal dysfunction.<sup>6 w5</sup> In addition, by blocking ET-1 generation, ECE inhibitors would act as mixed ET<sub>A</sub>/ET<sub>B</sub> antagonists and, perhaps importantly, would leave ET-1 clearance unaffected. However, so far, less progress has been made clinically with the development of drugs based on ECE inhibition than endothelin receptor antagonism.

#### Endothelin receptor antagonism

Endothelin receptor antagonists are classified as ET<sub>A</sub> or ET<sub>B</sub> selective, depending on their relative affinity for a receptor subtype, or mixed ET<sub>A</sub>/ET<sub>B</sub> antagonists when relatively non-selective (table 4). The two most used pharmacological "probes" to investigate the effects of endothelin receptor antagonism, either locally and systemically, are two peptides named BQ123 (antagonist of the ET<sub>A</sub> receptor) and BQ788 (antagonist of the ET<sub>B</sub> receptor). With regard to mixed ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists, a number of compounds have been used for experimental studies but bosentan (Tracleer), an orally available non-peptide antagonist, was the first of its class to obtain approval for clinical use from the US Food and Drug Administration and Europe's European Medicines Agency (EMA), and is currently licensed for the treatment of pulmonary arterial hypertension. It should be noted, however, that the distinction between selective and mixed (ET<sub>A</sub>/ET<sub>B</sub>) antagonists is not pharmacologically well defined. The mixed antagonists still usually demonstrate a greater affinity for the ET<sub>A</sub> receptor, whereas the ET<sub>A</sub> selective antagonists, when used at relatively high doses, may act on both receptors.<sup>w6</sup>

ET<sub>A</sub> receptor antagonism is associated with vasodilatation in healthy volunteers.<sup>5</sup> When administered systemically, the



**Figure 1** Factors regulating the synthesis of endothelin-1 (ET-1). ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C type natriuretic peptide; IL-1, interleukin-1; LDL, low density lipoprotein; TGF, tissue growth factor.

ET<sub>A</sub> receptor antagonist BQ123 produces a dose dependent reduction in blood pressure and systemic vascular resistance.<sup>w7</sup> This indicates that ET-1 contributes to the maintenance of vascular tone and blood pressure through its actions on the ET<sub>A</sub> receptor. In contrast, administration of the ET<sub>B</sub> receptor antagonist BQ788 produces a mild vasoconstriction and pressor effect.<sup>7,8</sup> This suggests that endothelial vasodilatory ET<sub>B</sub> receptors, known to be coupled to nitric oxide synthase, have a physiological role in counterbalancing ET<sub>A</sub> mediated vascular tone. Indeed, compared to isolated ET<sub>A</sub> receptor antagonism, mixed ET<sub>A</sub>/ET<sub>B</sub> receptor antagonism produces less vasodilatation and smaller falls in blood pressure.<sup>9</sup> ET<sub>B</sub> receptor antagonists have also been shown to increase plasma ET-1 concentrations in rodents and in man, presumably by decreasing ET-1 clearance.<sup>8, w8</sup>

In addition to its vascular effects at ET<sub>A</sub> and ET<sub>B</sub> receptors, ET-1 has a number of other identified physiological roles (fig 2). In particular, in the kidney, ET<sub>B</sub> receptors, apart from being present on endothelial cells where they cause vasodilatation, are also highly expressed in the renal tubular epithelial cells. Here the ET<sub>B</sub> receptors appear to respond to

intra-renal ET production preventing the actions of vasopressin and inhibiting Na/K-ATPase, which results in net salt and water loss.<sup>10</sup> This assumption is supported by the observation that ET<sub>B</sub> gene knockout mice have salt sensitive hypertension<sup>11</sup>; in addition, in the kidney, collecting duct derived ET-1 has been shown to be an important physiologic regulator of renal sodium excretion, as observed in collecting duct ET-1 knockout mice.<sup>w9</sup> ET-1 also plays a critical role in

**Table 3** Classification of endothelin converting enzyme inhibitors

Selective ECE inhibitors	Dual ECE/NEP inhibitors	Triple ECE/NEP/ACE inhibitors
<ul style="list-style-type: none"> <li>▶ Natural product</li> <li>FR 901532 (WS 79089B)</li> <li>B90063</li> <li>WS 75624B</li> </ul>	<ul style="list-style-type: none"> <li>▶ NEP&gt;ECE</li> <li>Phosphoramidon</li> <li>CGS 26303</li> <li>SLV 306</li> </ul>	<ul style="list-style-type: none"> <li>▶ Potent</li> <li>SCH 54470</li> </ul>
<ul style="list-style-type: none"> <li>▶ Synthetic compound</li> <li>SM 19712</li> <li>PD 069185</li> <li>CGS 35066</li> </ul>	<ul style="list-style-type: none"> <li>▶ ECE&gt;NEP</li> <li>CGS 34043</li> </ul>	<ul style="list-style-type: none"> <li>▶ Moderate</li> <li>SA 6817</li> <li>CGS26582</li> </ul>

ACE, angiotensin converting enzyme; ECE, endothelin converting enzyme; NEP, neutral endopeptidase.

**Table 4** Peptide and non-peptide ET receptor antagonists\*

ET receptor selectivity†		
ET <sub>A</sub>	ET <sub>B</sub>	ET <sub>A</sub> /ET <sub>B</sub>
<i>Peptide antagonists</i>		
BQ123	IRL2500	TAK044
BQ485	RES7011	PD142893
BQ153	BQ788	PD145065
BQ610		
FR139317		
PD151242		
<i>Non-peptide antagonists in clinical development</i>		
Darusentan (approx 150-fold ET <sub>A</sub> selective v ET <sub>B</sub> )		Enrasentan
		Tezosentan (iv)
Ambrisentan (approx 260-fold ET <sub>A</sub> selective v ET <sub>B</sub> )		
Atrasentan (approx 1860-fold ET <sub>A</sub> selective v ET <sub>B</sub> )		Bosentan
Sitaxsentan (approx 6500-fold ET <sub>A</sub> selective v ET <sub>B</sub> )		

\*The peptide compounds initially developed were subject to hydrolysatation and subsequent inactivation, by peptidases in the gastrointestinal and circulatory system, and orally available, non-peptide antagonists are currently the main focus of clinical research.

†ET<sub>A</sub> selectivity is generally taken as >100-fold selectivity for the ET<sub>A</sub> over the ET<sub>B</sub> receptors and ET<sub>B</sub> selectivity as 10–100-fold selectivity for the ET<sub>B</sub> over the ET<sub>A</sub> receptors. Davenport AP. International Union of Pharmacology. XXIX. Update on endothelin receptor nomenclature. *Pharmacol Rev* 2002;**54**:219–6.

embryonic development through both ET<sub>A</sub> and ET<sub>B</sub> receptors.<sup>12</sup>

### THERAPEUTIC INTERVENTION

Experimental and preclinical studies have highlighted the powerful actions of ET-1 as a vasoconstrictor, growth promoter, and pro-inflammatory agent, as well as a potential therapeutic role for endothelin antagonists in different cardiovascular and non-cardiovascular diseases. In particular, patients with pulmonary hypertension, heart failure, as well as arterial hypertension, renal dysfunction, and atherosclerosis, might benefit from treatment with endothelin antagonists.

#### Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a condition characterised by sustained elevation of pulmonary arterial pressure, caused by progressive obliteration of the pulmonary vascular bed, leading to increasing dyspnoea on exertion, fatigue, and progression to right ventricular failure.<sup>w10</sup> This condition has a poor prognosis, despite current treatment with oxygen therapy, diuretics, digoxin, and vasodilators. Until recently, the only agent specifically licensed for the treatment of severe PAH (World Health Organization functional classes III and IV) was epoprostenol, which is given by continuous intravenous infusion via an indwelling catheter: its administration is associated with risks of serious complications (infection or catheter correlated thrombosis, rebound hypertension).

#### Endothelin antagonism in PAH

PAH is associated with endothelial dysfunction, which results in impaired vasodilatation, exaggerated vasoconstriction, and activation of the endothelin system.<sup>w11</sup> The role of ET-1 in PAH is supported by various studies,<sup>13 w12</sup> and these findings have led to a rational therapeutic approach provided by endothelin receptor antagonists. In 2001 an early randomised, placebo controlled trial evaluated the effects of bosentan on exercise capacity and cardiopulmonary haemodynamics in 32 PAH patients, as well as its safety and tolerability. Bosentan improved exercise capacity as assessed by an increase in the six minute walking distance; in addition, nine out of the 21 bosentan treated patients improved their WHO functional class from III to II compared with only one of the 11 controls.<sup>14</sup>

The subsequent double blind, placebo controlled, multicentre BREATHE-1 (bosentan randomised trial of endothelin receptor antagonist therapy for pulmonary hypertension) trial confirmed these encouraging preliminary findings.<sup>15</sup> In this study, 213 PAH patients were enrolled and randomly assigned to bosentan 125 or 250 mg twice daily. Bosentan improved exercise capacity and WHO functional class, and increased time to clinical worsening. However, although well tolerated, it produced raised liver enzymes in 5% of patients on 125 mg and in 14% of patients on 250 mg.

On the basis of the data derived from these two trials, bosentan was approved for the treatment of PAH in patients with grade III WHO functional status; in addition, a one year follow up study suggests a sustained benefit on exercise capacity and haemodynamics.<sup>w13</sup> A selective ET<sub>A</sub> antagonist, sitaxsentan, is also currently being investigated for the treatment of PAH, after the preliminary positive results observed in the STRIDE-1 (sitaxsentan to relieve impaired exercise in pulmonary arterial hypertension) trial.<sup>w14</sup>

#### Unwanted effects

The clinical use of bosentan is associated with some safety issues. The most serious adverse event reported is a dose related hepatotoxicity. It occurs with elevations in aminotransferase, in most cases without any symptoms and more commonly when higher doses of bosentan were used. Its administration is also contraindicated in patients receiving glibenclamide and cyclosporine A. Other side effects include headache, flushing, and hypotension, and are usually of mild to moderate intensity. Decrease in haemoglobin concentration has also been reported. In addition, studies in animals have shown reproductive toxicity, including teratogenicity and embryotoxicity. For these reasons, the EMEA has recommended ongoing post-marketing surveillance.

Although the safety issues impose a careful monitoring of the therapy, the benefits shown in clinical trials, together with the ease of administration (125 mg twice daily oral dosing), suggest that bosentan may be a valid option for treatment of PAH patients of WHO functional class III, before considering intravenous infusion of epoprostenol (AJ Peacock, personal communication, 2004).

#### Heart failure

Chronic heart failure (CHF) is a major cause of cardiovascular mortality and morbidity. In most cases it is characterised by low cardiac output, leading to progressive haemodynamic and neurohumoral modifications, such as peripheral vasoconstriction, salt and water retention, and activation of the sympathetic and renin-angiotensin systems (RAS).<sup>w15</sup>

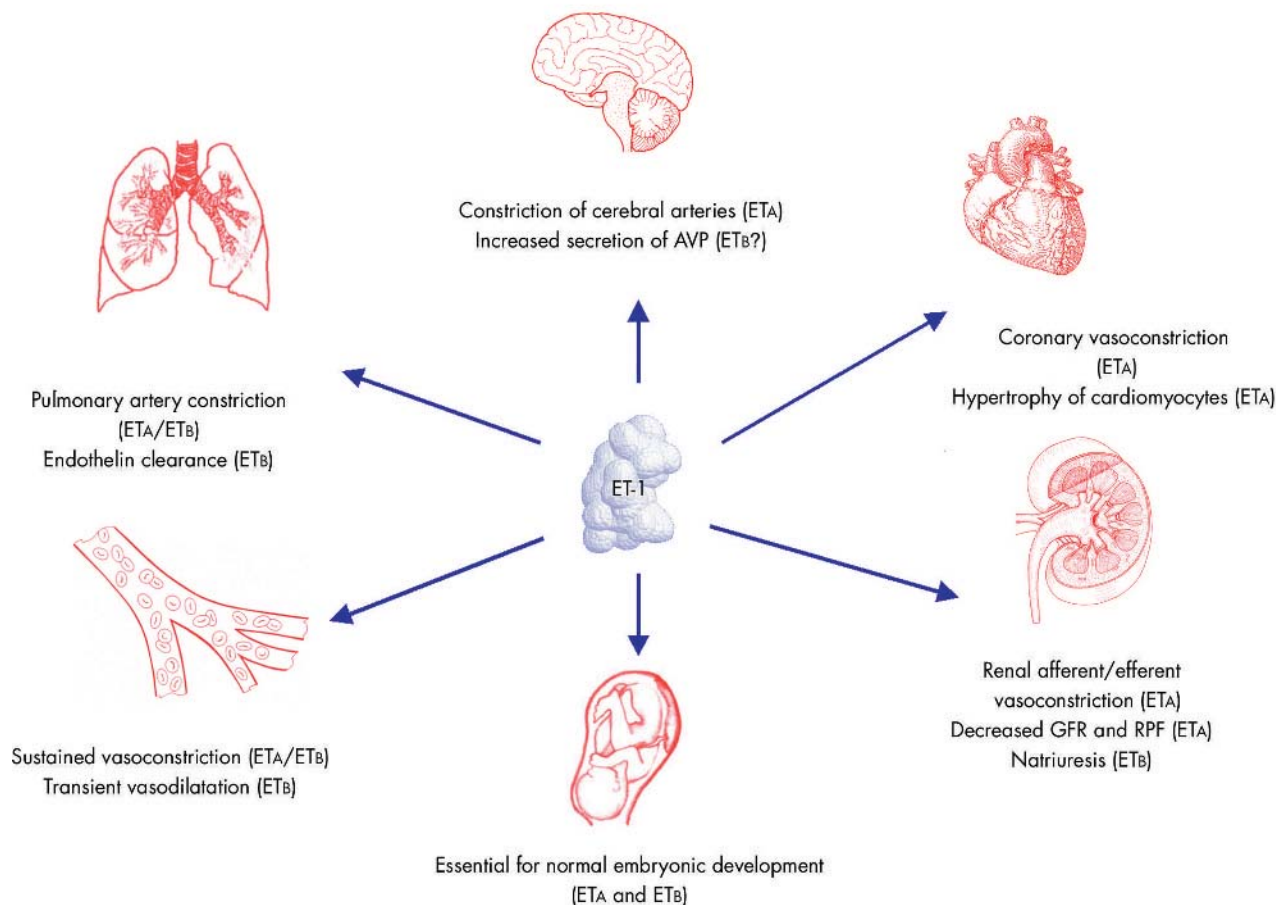
The endothelin system is activated in patients with CHF, as with other neurohumoral systems. Plasma big ET-1 and ET-1 concentrations have been correlated with clinical and haemodynamic measures of severity in patients with CHF and inversely with prognosis.<sup>16 17 w16 w17</sup> In addition, while ET-1 seems to exert a positive inotropic effect in the normal heart, an increase in myocardial contractility following ET<sub>A</sub> receptor blockade has been reported in the failing heart, suggesting that ET-1 may have a negative inotropic effect.<sup>w18</sup>

A number of large phase III studies of selective ET<sub>A</sub> and mixed ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists have now been completed in both acute heart failure (AHF) and CHF. Although initial experimental and acute haemodynamic studies were favourable,<sup>18 w19 w20</sup> the available information suggests that no clinical benefits derive from the use of endothelin antagonists in these conditions.

#### Clinical trials in acute and chronic heart failure

The RITZ project (randomised intravenous tezosentan) consisted of a group of four double blind, randomised and placebo controlled trials, assessing the effects of the intravenous mixed ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist tezosentan in AHF. Apart from RITZ-2, in which tezosentan, at a dose of 50 mg/hour, significantly improved cardiac index and reduced pulmonary capillary wedge pressure with a good safety profile,<sup>w21</sup> the other trials failed to demonstrate any beneficial effect of endothelin antagonism.

With regard to CHF, the first phase III study to be reported was the ENCOR (enrasentan clinical outcomes randomised) trial, which used the oral mixed ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist, enrasentan. Enrasentan treatment did not improve clinical status in these patients. In addition, it was associated with three times the hospitalisation rate, a trend towards greater mortality, and was not well tolerated. The subsequent



**Figure 2** Schematic representation of endothelin-1 (ET-1) actions. AVP, arginine vasopressin; GFR, glomerular filtration rate; RPF, renal plasma flow.

ENABLE (I/II) (endothelin antagonist bosentan for lowering cardiac events) and EARTH (endothelin antagonist receptor trial in heart failure) trials have also shown disappointing results.

Overall, interest in the possible use of endothelin antagonists in heart failure has now considerably declined. The isolated positive results of RITZ-2 study may hold out some promise for those who believe that endothelin antagonists may still have a therapeutic role in AHF, once the right timing of the intervention and the correct dose are identified. Experimental evidence from animal models suggests that early initiation of a mixed  $ET_A/ET_B$  receptor antagonist post-myocardial infarction leads to adverse effect and poor outcome.<sup>w22</sup> These data add on to recent studies in CHF animal models, where early use of an  $ET_A$  receptor antagonist causes further activation of the RAS and, in addition, results in sodium retention, without offering any substantial benefit.<sup>w23</sup> Better results may also be obtained by selecting those patients with a high degree of pulmonary hypertension.<sup>w20</sup> Whether combined ECE and NEP inhibition would provide a more effective intervention than endothelin receptor antagonism also remains to be established.

### NEW THERAPEUTIC PERSPECTIVES

Heart failure and pulmonary hypertension are not the only therapeutic areas in which endothelin antagonism has been investigated. Currently, there are interesting possibilities, in

terms of potential benefit, in the treatment of both arterial hypertension and chronic renal failure (CRF); in addition, animal studies suggest a role for therapeutic endothelin antagonism in atherosclerosis.

### Arterial hypertension

A number of studies have investigated the possible role played by endothelin in arterial hypertension. Certain animal models of experimental hypertension, in particular the salt sensitive and low renin forms, are associated with enhanced activity of ET-1.<sup>19</sup> Data from different groups suggest increased vascular endothelin activity in patients with essential hypertension, as manifested by greater forearm vasodilatation following the infusion of endothelin receptor antagonists, compared to normotensive subjects.<sup>w24 w25</sup> In addition, black hypertensive patients, who are often characterised by low renin values, have been shown to have higher plasma ET-1 concentrations than white hypertensives and to have enhanced  $ET_A$ -dependent vasoconstrictor tone.<sup>w26 w27</sup>

One of the first studies evaluating the effects of systemic endothelin antagonism on blood pressure was published in 1998: in a cohort of 293 patients with essential hypertension, the effects of bosentan and enalapril on blood pressure were compared. Bosentan significantly lowered diastolic blood pressure and its effect was similar to enalapril. In addition there was no concomitant reflex activation of the RAS or the

sympathetic nervous system.<sup>20</sup> In another study conducted in hypertensive patients, darusentan, an oral selective ET<sub>A</sub> receptor antagonist, was efficacious in lowering both systolic and diastolic blood pressure, compared to the placebo group.<sup>w28</sup> Recently, a new antagonist, with dual angiotensin (AT<sub>1</sub>) and ET<sub>A</sub> receptor actions, has been shown to reduce blood pressure in an experimental model of hypertension.<sup>w29</sup> On the basis of these data, although endothelin antagonism is unlikely to be considered as first line treatment, it could still have a role in the future in the treatment of high risk patients, such as black hypertensives or patients with resistant hypertension, after an accurate risk/benefit assessment.

### Chronic renal failure

A large body of experimental evidence suggests the involvement of ET-1 in the pathophysiology of chronic renal failure (CRF), and endothelin antagonists have been shown to improve renal function in experimental models of kidney disease.<sup>3</sup> <sup>w30</sup> The results of a randomised, placebo controlled study recently conducted in hypertensive subjects with CRF highlighted the potential benefits on blood pressure and renal function that can be achieved with endothelin antagonism. This study aimed at comparing the effects of ET<sub>A</sub>, ET<sub>B</sub>, and mixed ET<sub>A</sub>/ET<sub>B</sub> antagonism in CRF patients and matched healthy controls. In CRF patients, the selective ET<sub>A</sub> receptor antagonist BQ123 lowered systemic blood pressure > 10 mm Hg compared with placebo, increased renal blood flow, and reduced renal vascular resistance.<sup>9</sup> Another benefit, which may derive from the use of endothelin antagonism in renal failure, is related to the possible involvement of ET-1 in parathyroid cell proliferation *in vivo*, which may influence the development of secondary hyperparathyroidism: data from experimental studies suggest that bosentan is able to prevent parathyroid cell proliferation in rats.<sup>w31</sup> Given the need for better and more effective strategies to prevent the consequences of secondary hyperparathyroidism in chronic renal failure, such as renal osteodystrophy, endothelin antagonism may offer additional benefits in this class of patients. Last, but not least, teratogenicity is less likely to represent an issue in this particular class of patients.

### Atherosclerosis

ET-1 seems to be involved from an early stage in the development of atherosclerosis. Increased plasma ET-1 concentrations have been demonstrated in patients with cardiovascular risk factors such as hypercholesterolaemia, hypertension, and diabetes mellitus.<sup>w32</sup> In addition, increased expression of ET-1 and ECE has been shown in endothelial cells, vascular smooth muscle cells, and macrophages at different stages of atherosclerotic plaque evolution.<sup>w33</sup> In these conditions, endothelial dysfunction (ED) develops early on. ED is characterised by an imbalance between vasoconstrictor (pro-inflammatory factors) and vasodilator factors, resulting in cell growth, inflammation and, ultimately, vascular remodelling. It is believed to represent a very early common step in the progression to overt atherosclerosis. Patients with established atherosclerosis have been shown to have a significant correlation between plasma ET-1 concentrations and the number of atherosclerotic lesions, and both ET<sub>A</sub> and ET<sub>B</sub> receptors are highly expressed in smooth muscle cells and foamy macrophages in atherosclerotic plaques.<sup>w34</sup> <sup>w35</sup> Increase in nitric oxide

### Endothelin antagonism: key points

- ▶ Endothelin-1 (ET-1) is a broadly active and extremely potent vasoconstrictor
- ▶ ET-1 is involved in the pathophysiology of pulmonary arterial hypertension, heart failure, systemic hypertension, renal dysfunction, and atherosclerosis
- ▶ The discovery of several compounds acting as endothelin antagonists has prompted research towards their use in clinical practice
- ▶ Bosentan is a combined ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist, and the first compound of its class to be approved and marketed for the treatment of pulmonary arterial hypertension
- ▶ Bosentan use requires careful monitoring due to the dose dependent liver toxicity and is contraindicated in pregnancy because of teratogenicity
- ▶ Endothelin antagonists in heart failure have shown disappointing results, the reasons for which are still unclear
- ▶ Endothelin antagonists also show therapeutic potential in chronic renal failure, arterial hypertension, and atherosclerosis

synthase (NOS) activity and improvement in NO mediated vasodilatation, as well as reduction in atherosclerotic lesion development, have been noted following endothelin antagonism in animal models of hypercholesterolaemia and atherosclerosis.<sup>w36</sup> <sup>w37</sup> Available data in atherosclerotic patients show an increased ET-1 mediated vascular tone, together with an enhanced forearm vasodilatation evoked by combined endothelin receptor blockade.<sup>21</sup> It has also been demonstrated that ET-1, acting through the ET<sub>A</sub> receptor, participates in the maintenance of basal human coronary vasoconstrictor tone and contributes to ED.<sup>w38</sup> These data support the role played by the ET system in the development of ED and the progression to atherosclerosis, and suggest a potential therapeutic role for endothelin antagonists in this scenario.

### OUTSTANDING ISSUES

Soon after its discovery, it became clear that ET-1 is involved in many cardiovascular diseases and a number of clinical trials with endothelin receptor antagonists have been undertaken. However, almost 15 years on, their clinical use is limited to bosentan in pulmonary arterial hypertension. Why have results been so disappointing, in spite of the encouraging experimental studies? To answer this question, and to provide a brief summary of the key issues discussed in this review, we need to focus on the following.

#### Doses used in clinical trials

Two points need to be considered: efficacy and safety. Initially, in the hope of better results, relatively high doses of endothelin antagonists were used, and there has been considerable debate as to whether the doses in these studies have been too high, leading to worse outcomes. Indeed, the use of high doses has been associated with higher incidence of unwanted effects, such as liver toxicity, and the propensity for fluid retention when on bosentan. It has been suggested that foreknowledge of this effect could have allowed optimal clinical management (that is, more aggressive concomitant diuretic therapy) and, therefore, safer use of the drug.

### Selectivity of endothelin antagonism

Whether selective ET<sub>A</sub> or combined ET<sub>A</sub>/ET<sub>B</sub> receptor antagonism may represent the best strategy for therapeutic modulation of ET receptors has been a matter of major controversy. The different actions of ET-1 on the ET<sub>A</sub> and ET<sub>B</sub> receptors on blood vessels, and the role played by ET<sub>B</sub> receptors in salt and water balance, endothelium dependent vasodilatation, and in the clearance of circulating ET-1, suggest that selective ET<sub>A</sub> antagonism might be the best approach. However, in disease states, these two receptors appear to be differently regulated and expressed, as evidenced by studies conducted in left ventricular systolic dysfunction and in atherosclerosis.<sup>w39 w40</sup> Taken together, these findings underline the complexity of the role played by ET-1 in cardiovascular disease, as well as the difficulty in knowing which receptor to target. For this reason, further studies elucidating the exact role played by endothelin receptors are warranted.

### Disease and disease state

ET-1 antagonism is effective in PAH, which is a serious but rare disease, whereas the overall effect in more common cardiovascular diseases may be considered modest, in spite of the promising results obtained in experimental studies. A possible explanation is that haemodynamic parameters may be a poor surrogate for clinical efficacy and do not necessarily convert into benefits for patients, and that what we know about one disease may not translate to another. Indeed, the poor outcome in CHF does not mean that there may not be benefits elsewhere.

### Teratogenicity

Although most of the available data are on bosentan, this is undoubtedly likely to be a class effect. As already mentioned, studies in animals have shown dose dependent teratogenic effects, including malformation of the head, mouth, face, and large blood vessels, which occur during the first trimester of pregnancy. This may limit the clinical impact of endothelin receptor antagonists and, overall, impose a strict and careful monitoring of the treatment.

### CONCLUSIONS

The development of endothelin antagonists presented a novel and potentially interesting therapeutic intervention in patients with cardiovascular disease. This class of drugs has proven its promise in pulmonary hypertension but failed, until now, to demonstrate clinical utility in patients with heart failure.

Whether combined ECE and NEP inhibitors will be more efficacious in the treatment of heart failure remains to be established but, undoubtedly, chronic renal failure, arterial hypertension, and atherosclerosis represent interesting research fields for future studies.

Additional references appear on the *Heart* website—<http://www.heartjnl.com/supplemental>

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Competing interest statement: Professor Webb has, in the past, provided advice to pharmaceutical companies (Abbott, Actelion, Astra Zeneca,

BMS, Encysive, Roche) on the clinical development of endothelin antagonists for cardiovascular disease.

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