New drug classes

Endothelin antagonists

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The very potent endogenous vasoconstrictor endothelin was discovered in 1988. We know now that there are three isoforms (1, 2, and 3) and two receptor subtypes (A and B). A whole range of peptide and non-peptide antagonists has been developed, some selective for A or B receptors and others with non-selective A/B antagonistic activity. So far the main application of these agents has been experimental—ie, endothelin blockers are used to throw light on disease mechanisms, most notably cardiovascular and renal. However, the non-selective antagonist bosentan and a few other agents have been studied clinically. Evidence so far from preclinical studies and healthy volunteers and from the limited number of investigations in patients permits a listing of the potential areas of clinical interest. These are mainly cardiovascular (eg, hypertension, cerebrovascular damage, and possibly heart failure) and renal. Clouds on the horizon are the need to show that these new agents are better than existing drugs; the possibility of conflicting actions if mixed A/B antagonists are used; and animal evidence hinting that endothelin blockade during development could be dangerous.

In a search for endogenous factors with vasoactive properties Yanagisawa and coworkers in 1988[1] found that porcine cells generated a 21-aminoacid peptide, endothelin, one of the most potent endogenous vasoconstrictors yet identified. Endothelin constricts coronary arteries in vitro and induces hypertension in laboratory animals and man. In the decade since 1988 knowledge about the biology of endothelins and the role of endothelins in disease has accumulated rapidly. The aim of this review is to summarise research into the pharmacological strategy of endothelial-receptor blockade—but before that we will outline what is known about the biology of endothelin and its receptors and about their role in disease.

Biology of endothelins

Synthetic pathway and breakdown

The human genome holds three distinct endothelin genes, on different chromosomes, encoding the closely related products ET-1, ET-2, and ET-3.[2] Endothelin peptides are produced within the cell as large preproendothelins; these are then cleaved at two sites by a neutral endopeptidase forming biologically inactive precursor "big endothelins",[1] which are ultimately converted to mature peptides by endothelin-converting enzyme(s) (figure 1).[1]

Endothelin is synthesised not only in the endothelium but also in the brain, lung, kidney, and some circulating cells. The pathway of endothelin synthesis is now well defined but its complex process of degradation, which also influences biological activity, is not. Diverse stimuli modulate endothelin expression and release, among which are transforming growth factor β, interleukin-1, angiotensin II, and fluid-mechanical shear stress.

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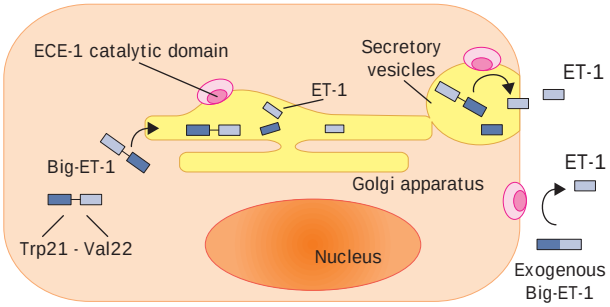

Figure 1: **Regulation of endothelin secretion**
Transfer of cDNA for endothelin-converting enzyme 1 (ECE-1) to Chinese hamster ovary cells that normally secrete only big-ET-1 revealed that ECE-1 is a potential regulatory site for production of active peptide. Intracellular form of ECE-1 converts endogenously produced ET-1 and is apparently localised in the Golgi apparatus. Another property of ECE-1 is an ectoenzyme expressed on the cell surface and capable of cleaving the big-ET-1 supplied from outside the cell.

ET receptors

Because ET is hydrophilic and thus unable to cross the plasma membrane, it must bind to specific cell-surface receptors whose expression will govern the cell response to the peptide. The existence of distinct endothelin isoforms with diverse function predicted the existence of distinct receptors. Two have been identified. Type A (ET_A) has a higher affinity for ET-1 and ET-2 and less for ET-3; ET_B binds all three isopeptides with nearly identical affinity.[3] ET_A receptors are found in vascular smooth-muscle cells and mediate vasoconstriction and cell proliferation. ET_B receptors on endothelial cells mediate vasodilation via nitric oxide. A variety of additional functions has been attributed to ET_B receptors, however, and in certain instances they can even elicit vessel contraction.[4] Moreover, the ET_B receptor initiates a positive autocrine loop by which ET-1 regulates expression of its own gene.[5] Coupling of activated receptors to effector systems generates the second messengers, which include inositol phosphates, diacylglycerols, and calcium and are ultimately responsible for the biological effects.

ET-producing tissues all express specific binding sites, suggesting a local rather than circulating hormone-type

Panel 1: **Endothelin receptor antagonists tested in animal models***

Disease	ET antagonist	Type
Cardiovascular		
Genetic hypertension	BQ 123	A
	A-127722·5	A
	LU 135252	A
	BMS-182874	A
	SB 209670	A / B
Pulmonary hypertension	BQ 123	A
	LU 135252	A
	A-127722	A
	Bosentan	A / B
	SB 209670	A / B
	BMS 182874	A
Congestive heart failure	BQ 123	A
	Bosentan	A / B
	PD 156707	A
Myocardial infarction	BQ 123	A
	Bosentan	A / B
Arteriosclerosis	SB 209670	A / B
	Bosentan	A / B
Cerebrovascular		
Subarachnoid haemorrhage	BQ 123	A
	RO 47-0203	A / B
	BQ 485	A
	FR 139317	A
	Bosentan	A / B
	SB 209670	A / B
	PD 145065	A / B
	BQ 610	A
Cerebrovascular (cont'd)		
Cerebral ischaemia	BQ 123	A
	Bosentan	A / B
	SB 217242	A / B
	PD 155080	A
Renal		
Renal ischaemia	BQ 123	A
	SB 209670	A / B
	L-754142	
Toxic nephropathies	BQ 123	A
	SB 209670	A / B
	RO 470203	A / B
	Bosentan	A / B
	CP 170687	A / B
Transplantation	BQ 123	A
Progressive nephropathies	FR 139317	A
	BMS 182874	A
	Bosentan	A / B
	RO 462005	A / B
	FR 139317	A
	PD 142893	A / B
	LU 135252	A

*For key references, see text.

regulation. Plasma concentrations of endothelins are in the picomolar range, far below the pharmacological threshold. Specific binding sites have been identified in several fetal and adult organs, including lung, heart, brain, and kidney.[6] In rat cardiac atrium ET_A is the major receptor type while in brain the ET_B receptor seems predominant.[6] The relative abundance of A and B receptors in the kidney depends on species; the ET_A/ET_B ratio approaches unity in rats but in man ET_B is more prominent.[6]

Effects of endothelin and role in disease

Some of what has been learned about the effects of exogenous endothelin (see below) and much of our knowledge about endothelin and the pathophysiology of disease has come from research with ET antagonists. These aspects can be considered only briefly here.

Bolus injections of nanomolar amounts of ET-1, ET-2, and ET-3 in laboratory animals lower blood pressure by the transient induction of prostacyclin and nitric oxide release from endothelial cells when the endothelin binds to specific receptors. In man, when large concentrations of ET-1 or ET-3 are given into the forearm circulation, transient vasodilation occurs before sustained vasoconstriction, consistent with an effect on the ET_B receptor. The dose-dependent remarkable increase in systemic blood pressure[1,2] which follows is prolonged and mostly dependent on renal, mesenteric, and muscular vessel constriction; the pulmonary circulation is far less sensitive. Endothelin of central-nervous-system origin may also enhance arterial pressure.

The systemic, but not renal, pressor effect of ET-1 in rats was abolished by the selective ET_A antagonist BQ 123[7] but both systemic and renal vasoconstriction were prevented by the ET_A/ET_B antagonist PD 145065, suggesting a role for the ET_B receptor in the renal response to ET-1.[8] ET-1 infusion causes profound renal vasoconstriction in healthy volunteers but systemic blood pressure is hardly affected.[9] Human data, taken together, point to a unique susceptibility of renal vessels to ET-1 vasoconstriction.

Pathophysiology derived from ET receptor antagonists (panel 1)

Hypertension

Normalisation of blood pressure in genetic models[10–15] by ET_A or ET_A/ET_B receptor blockade might suggest that endothelin participates in the pathophysiology of hypertension but this is difficult to reconcile with the finding that ET_A knockout mice are hypertensive.[16] Interpretation of the experimental findings is further complicated by evidence that endogenous overexpression of preproET-1 increases plasma ET-1 levels to values found in disease and causes systemic hypertension through activation of the ET_A receptor.[17]

Human data are also conflicting. In support of a role of endothelin in hypertension is a report of two patients with a rare vascular neoplasm of endothelial cell proliferation, who had systemic hypertension and raised circulating endothelin concentrations.[18] After removal of the tumour the blood pressure decreased and endothelin levels became normal while tumour recurrence in one patient was accompanied by a return to high blood pressure and enhanced circulating endothelin. However, more common causes of systemic hypertension are not clearly linked to high circulating endothelin.[19] Endothelin acts as an autocrine/paracrine substance on the underlying vascular smooth muscle, where its concentration may be several orders of magnitude higher than it is in plasma. For instance, in atherosclerosis vascular endothelin increases remarkably in the face of a slight increase in endothelin in the peripheral blood.[20] ET-1 induces expression of several

Panel 2: **Evidence for role of endothelin in rat models of acute renal failure induced by ischaemic/reperfusion injury**

Endothelin antagonism		Outcome
Type	Agent	
Antibody	Anti-ET-Ab	GFR decline showed[82]
ET_A antagonist	BQ123	Lower serum creatinine[83]
ET_A/ET_B antagonist	TAK-044	Lower serum creatinine[84]
ET_A/ET_B antagonist	RO 46-2005	Reduced renal vasoconstriction[85]
ET_A antagonist	BQ123	Increased sodium reabsorption[86]
ET_A/ET_B antagonist	SB 209670	Normal GFR[87]

protooncogenes that can promote smooth-muscle-cell proliferation and the ET_A receptor antagonist BQ 123 prevented ET-1-induced mitogenesis in rat smooth-muscle cells.[21] One ET_A/ET_B receptor antagonist (SB209670) protected rat carotid artery from neointimal formation after balloon angioplasty[22] while another (bosentan) prevented graft arteriosclerosis in rat cardiac allograft.[23]

Heart failure

In two animal models of congestive heart failure plasma ET-1 is increased and the changes correlate with right atrial and pulmonary capillary wedge pressures. In the rat model the ET_A receptor antagonist BQ 123 ameliorated left-ventricular function and improved survival.[24] A benefit from chronic ET_A receptor blockade was also seen in the rabbit model.[25] In rats with chronic heart failure bosentan reduced systemic vasoconstriction and lowered blood pressure.[26] Thus the endothelin system does seem to be implicated in the complex mechanisms leading to end-stage organ damage in chronic heart failure.

In patients with congestive heart failure high ET-1 plasma levels correlate with left-ventricular end-diastolic volume, left atrial pressure and pulmonary hypertension,[27–29] as in animals.

Myocardial infarction

High plasma ET levels have been reported in experimental ischaemia.[30] When given to rats after myocardial infarction, endothelin-receptor antagonists improved left-ventricular function, attenuated the development of pulmonary hypertension, and increased survival.[24,31,32] In patients with myocardial infarction plasma levels of ET-1 are very high[33] and ET-1 concentrations in plasma predict 1-year mortality.[34]

Pulmonary hypertension

Rats exposed to hypoxia or monocrotaline display increased pulmonary artery pressure and right-ventricular hypertrophy associated with high plasma ET-1 levels and selective enhancement of ET-1 peptide and ET-1 and ET_A mRNA in the lung. Both were prevented or at least retarded by ET_A and ET_A/ET_B receptor antagonists.[35–39] Venous plasma ET-1 levels are raised in patients with pulmonary hypertension,[40] as is expression of ET-1 in vascular endothelial cells of the lung.[41]

Cerebrovascular disease

Raised concentrations of ET-1 and ET-3 have been reported in cerebrospinal fluid of animals and of patients with cerebral haemorrhage, and ET_A or ET_A/ET_B receptor antagonists are protective in animal models of stroke.[39,42,43] In the normotensive rat neither BQ 123 nor bosentan altered ischaemic damage or cerebral blood flow after focal ischaemia but in the spontaneous hypertensive rat BQ 123 and SB 217242 reduced the volume of ischaemic damage by about a quarter.

Renal disease

Some of the evidence for a role for endothelin in post-ischaemic acute renal failure is circumstantial but direct evidence comes from studies with endothelin antibody or receptor antagonists (panel 2). In dogs endothelin-receptor antagonists improved glomerular filtration, renal blood flow, and sodium excretion after renal artery or abdominal aortic occlusion.[44,45] This has been confirmed in kidney transplantation; in rats BQ 123 and bosentan protected kidneys from the cold ischaemia-reperfusion damage.[46,47]

ET-1 mediates, at least in part, acute cyclosporin-induced renal vasoconstriction and this drug and related immunosuppressive drugs all enhance the release of endothelin from vascular endothelial cells and up-regulate renal endothelin receptors. An anti-ET antibody prevented cyclosporin-associated renal hypoperfusion in the rat and ET_A or ET_A/ET_B receptor antagonists ameliorated or even prevented acute cyclosporin-induced renal vasoconstrition.[48–50] ET-1 may have a role in chronic cyclosporin nephrotoxicity too.[51] ET-1 may also be involved in radiocontrast nephropathy and ET_A and ET_A/ET_B receptor antagonists are protective.[52–54]

Bosentan protected against glycerol-induced renal failure, a model for rhabdomyolysis,[55] and ET-receptor blockade ameliorated the renal vasoconstriction induced by endotoxin.

There is indirect evidence for a role for ET-1 in other forms of renal damage—eg, in rats with passive Heymann nephritis, an immune model resembling membranous nephropathy; in NZB/WF1 mice with an immunological disease reminiscent of human lupus; and in experimental diabetes. More direct evidence comes from transgenic animals. Mice overexpressing the human ET-1 promoter form more ET-1 in their kidneys and develop renal lesions despite no increase in systemic blood pressure.[56] Rats transgenic for the human ET-2 gene are normotensive but have renal lesions reminiscent of those seen in the remnant kidney rat model.[57]

In the rat remnant kidney model ET-receptor antagonists ameliorated renal function and protected against glomerular and tubulointerstitial structural injury.[58–60] By contrast, endothelin-receptor antagonists that consistently reduce renal damage do not always normalise proteinuria, strengthening the possibility that excessive ET-1 is not the cause but a consequence of increased glomerular protein traffic. The renoprotective effect of FR 139317 was also apparent in mice with experimental lupus nephritis[61] and in streptozotocin-induced diabetes in rats. A peptidic unselective ET_A/ET_B receptor antagonist (PD 142893), when chronically administered to diabetic animals with overt proteinuria, was as effective as an angiotensin-converting enzyme (ACE) inhibitor on blood pressure and protein excretion.[62]

Endothelin antagonists in clinical practice

The first endothelin antagonist developed for human testing was a natural byproduct of the fermentation of *Streptomyces misakiensis* but this had low potency in binding and functional assays. Subsequently, a series of peptide receptor antagonists selective for ET_A or ET_B or

non-selective (ET_A/ET_B) were developed (panel 3).[42,63–66] However, these compounds are hydrolysed by peptidase in the systemic circulation and gastrointestinal tract; nor do they penetrate the blood-brain barrier when given systemically. Recently, non-peptide antagonists have been developed. The first was Ro 46-2005, a non-selective ET_A/ET_B receptor blocker.

Compounds already under test clinically

Few clinical studies have been done with endothelin antagonists (figure 2). In healthy volunteers brachial artery infusion with BQ 123 caused progressive forearm vasodilation[67] and a placebo-controlled crossover study in healthy volunteers also found that systemic ET_A/ET_B receptor blockade with TAK-044 lowered peripheral vascular resistance and blood pressure.[68] Increased forearm blood flow has also been reported in patients with chronic heart failure treated with ACE inhibitors after local infusion of BQ 123.[69] Bosentan (100 mg followed 1 h later by 200 mg) lowered pulmonary artery pressure and vascular resistance in patients with chronic heart failure and increased cardiac index.[70] Whether this heralds a new indication for this class of compounds in heart failure cannot be resolved by study of acute haemodynamic changes but requires formal long-term evaluation of morbidity and mortality.

In a recent study 293 patients with mild-to-moderate essential hypertension were randomly assigned to different oral doses of bosentan for 4 weeks.[71] Bosentan significantly lowered blood pressure with no changes in heart rate and without activation of the sympathetic nervous system or renin-angiotensin system. To show that endothelin contributes causally to essential hypertension requires exclusion of the possibility that bosentan interferes with a physiological function of endothelin in maintaining normal blood pressure.[72] Endothelin-receptor antagonists have been generally poorly effective in lowering systemic blood pressure but a role for them in preventing the effect of hypertension on end-organ damage cannot be ruled out. Bosentan is undergoing clinical trials in subarachnoid haemorrhage.[73] A recent study in 10 healthy volunteers found that bosentan limited cyclosporin-induced renal hypoperfusion without effects on the systemic blood pressure.[74]

Despite the paucity of clinical studies the hope remains that ET-receptor antagonists will offer benefit to patients with hypertension and cardiovascular diseases, especially those with severe neurohumoral vasoconstriction (possibly including chronic heart failure). However, a multicentre double-blind randomised trial in high-risk patients undergoing coronary angiography showed that intravenous administration of the ET_A/ET_B receptor antagonist SB 209670 did not prevent the nephrotoxicity that follows radiocontrast administration, questioning the validity of endothelin-receptor antagonism prophylaxis in these patients.[75]

Panel 3: **Peptide and non-peptide endothelin-receptor antagonists**

ET receptor selectivity		
ET_A	ET_B	$ET_A ET_B$
Peptide molecules		
BQ 123	BQ 788	PD 142893
BQ 485	IRL 1038	PD 145065
BQ 610		TAK-044
FR 139317		
Non-peptide molecules		
PD 155080		Bosentan
PD 156707		RO 46-2005
BMS 182874		L-754142
A-127722		L-751281
		SB 209670
		SB 217242
		RO 470203
		CP 170687
		LU 135252

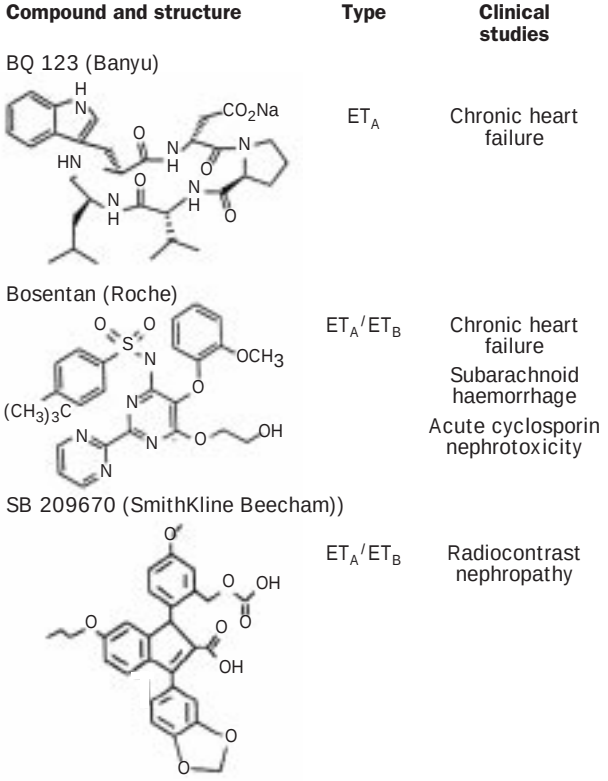

Figure 2: **ET-receptor antagonists under clinical development**

Molecules earlier in development

In this section of our review we have concentrated on compounds that have been studied in man, either in volunteers or clinically. However, at least twelve drug companies have a research programme in endothelin-receptor antagonists in the preclinical phase of development.[76]

Future possibilities and problems

The preclinical studies point to a role for endothelin-receptor antagonists in cardiovascular diseases (including hypertension and cerebrovascular damage) and in renal dysfunction. Patients with essential hypertension might benefit but the advantages, if any, of this drug class over conventional antihypertensive therapy are unknown. Other possibilities are chronic heart failure, pulmonary hypertension, symptomatic atherosclerosis, subarachnoid haemorrhage, and the prevention of progressively deteriorating renal function in proteinuric nephropathies, including the nephropathy associated with diabetes.

Before we move from preclinical pointers to clinical studies there are concerns that must be addressed. Blockade of ET_A receptors alone may be different from blocking ET_B or ET_A/ET_B receptors. If activation of the ET_B receptor has a nett hypotensive effect, for instance, ET_A receptor antagonism may be contraindicated in

conditions associated with low blood pressure, such as sepsis. Selective ET_B receptor blockade, on the other hand, could theoretically worsen ET_A-induced renal vasoconstriction. And, also on the safety side, major abnormalities in animals rendered genetically deficient of endothelin or of endothelin receptors indicate that inhibition of endothelin during development could be devastating. Mice homozygous for ET-1 mutation have cardiovascular malformations and abnormalities of craniofacial organs derived from the pharyngeal arch,[77] indicating that ET-1 is essential to normal embryonic development. Mice "knocked out" for ET_A receptor develop lesions[78] that mimic those seen in the velocardiofacial and CATCH 22 (cardiac anomaly, abnormal face, thymic hypoplasia, cleft palate, hypocalcaemia, chromosome 22 deletions) syndromes in man. Mice deficient in ET-3 or the ET_B receptor[79] develop white spotted coats and aganglionic megacolon due to absence of neural-crest-derived melanocytes and enteric neurons, a phenotype overlapping with that of patients with Hirschsprung disease, in which mutations of the ET-3 and ET_B receptor genes have been reported.[80,81]

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