Endothelin: from molecule to man

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Background

Until relatively recently, the vascular endothelium was thought to act as little more than a passive barrier to diffusion. However, in the last few years, with the discovery of prostacyclin, nitric oxide and endothelin-1, a central role has emerged for the endothelium in the regulation of vascular smooth muscle cell tonus and growth, immunological reactivity, blood coagulation and lipid metabolism. This field of research has matured rapidly (Figure 1) and, in a recent foresight exercise on behalf of the British Heart Foundation, Medical Research Council and Wellcome Trust [1], the vascular endothelium was identified as the area commanding the highest priority in cardiovascular research over the next 10 years.

My own interest in this area originally developed while working in the MRC Blood Pressure Unit in Glasgow. Observations in the first clinical trial of a renin inhibitor in man suggested the functional importance of a vascular renin-angiotensin system [2] and, later, at St George's Hospital in London I was able to show this by combining forearm plethysmography techniques with brachial artery infusions of locally active doses of angiotensin I, angiotensin II, bradykinin and angiotensin converting enzyme (ACE) inhibitors [3, 4]. Surprisingly, these studies demonstrated *in vivo* that the endothelium of the forearm resistance vessels has a considerable capacity to generate angiotensin II locally, equivalent to that of the pulmonary circulation [4, 5].

The discovery in 1988 of the novel 21 amino acid

peptide, endothelin-1 [6], a more potent endothelium-derived vasoconstrictor and pressor agent even than angiotensin II, was of major interest to the cardiovascular research community, and followed closely on the identification of the endothelium-derived relaxing factor (EDRF) as nitric oxide [7]. At this early stage it was difficult to gain access to more than small quantities of synthetic endothelin-1. However, it was clear that local infusion techniques could allow delineation of the vascular effects of endothelin-1 more clearly, with greater safety, and with lower doses, than would be the case with systemic dosing. In collaboration with Dr John Clarke and Professor Attilio Maseri at the Royal Postgraduate Medical School, we began clinical pharmacology studies at St George's Hospital in London in 1988, and our first pharmacodynamic observations were published the following year [8].

Subsequent progress in endothelin research [9, 10] has maintained an extremely rapid pace such that we were able to perform the first human pharmacology studies with an inhibitor of endothelin generation, phosphoramidon, in 1992 and with an endothelin receptor antagonist, BQ-123, in 1993. Indeed, after only 8 years, the clinical development of drugs targeting the 'endothelin system' in cardiovascular disease is now well advanced [11]. The first major clinical study in heart failure patients was reported in 1995 [12] and phase III trials should begin shortly. From a combination of fundamental and applied research it has become apparent that the 'endothelin system' is of central importance to the maintenance of normal cardiovascular function in healthy man and that endothelin-1 is likely to be a key mediator in the pathophysiology of cardiovascular disease.

The main aim of this paper is to review current knowledge of the endothelin system; particularly its physiological function in the cardiovascular system and its role in cardiovascular disease. Although I will focus on human pharmacology and physiology, and in particular on the work of our group in Edinburgh, it must be recognised that all of the major advances in clinical research have depended critically on the identification and development of selective pharmacological probes through work on animal and isolated tissues. A subsidiary aim of this review is to demonstrate that clinical pharmacology studies can provide a critical contribution to our understanding of human cardiovascular physiology and pathophysiology, and to the process of early drug development and identification of suitable therapeutic targets.

Figure 1 Annual numbers of papers in the biomedical sciences including the word 'endothelium' in the title, keywords or abstract from 1980 to 1996 inclusive; based on information from the Bath Information and Database Service (BIDS) EMBASE.

Endothelin synthesis

Endothelin-1 is the most potent vasoconstrictor and pressor peptide known. Originally identified in the culture medium

Figure 2 Structure of the endothelin family of peptides, and the related snake venom peptide sarafotoxin S6c.

of porcine aortic endothelial cells [6], it is now recognised to be a member of a family comprising three isoforms [13]: endothelin-1, endothelin-2 and endothelin-3 (Figure 2). Each isoform contains 21 amino-acids, two intra-chain disulphide bonds constraining overall structure, and a conserved C-terminal sequence necessary for biological activity [13]. This structure is unique among the mammalian peptides but is shared by the sarafotoxins, snake venom peptides from the Israeli burrowing asp, *Atractaspis engadensis*, one of which, sarafotoxin S6c, has proved particularly valuable as a pharmacological probe. While there is evidence that endothelin-2 may possibly function as a mediator in the kidney, and endothelin-3 may act as mediator in the gut and nervous system, endothelin-1 is the major isoform generated in blood vessels and appears to be of greatest significance in cardiovascular regulation. Hence, endothelin-1 is the major focus of this review.

Within the human genome, the endothelins are each represented by a separate gene encoding a specific precursor for the mature isoform [13]. In the 5' flanking sequence there are binding sites for activating protein-1 and nuclear factor 1 through which angiotensin II and transforming growth factor β act respectively to induce endothelin-1 expression. There are also binding sites for acute phase reactants which may mediate the effects of acute physiological stress. In the 3' region there is a sequence regulating selective destabilisation of preproendothelin-1 mRNA, possibly accounting for its short half-life. These sites serve as potential mechanisms for regulating production of endothelin at the level of transcription and translation. Although endothelin-1 can be identified within endothelial cells, it

remains unclear whether intracellular stored peptide represents an important pool available for rapid release and, currently, regulation of endothelin synthesis is thought to be primarily at the level of gene transcription, with *de novo* production and release occurring in response to endothelial cell stimulation. A large number of factors have now been shown to increase endothelin-1 synthesis (Figure 3): these include vasoactive hormones, inflammatory mediators and physico-chemical factors such as altered vascular shear stress and hypoxia. Other factors—including nitric oxide, nitric oxide donor drugs, natriuretic peptides and the dilator prostanoids—serve to inhibit endothelin-1 generation by promoting production of cyclic GMP or cyclic AMP [10].

The initial product of the human endothelin-1 gene is preproendothelin-1, a peptide of 212 amino acid residues (Figure 3). After removal of a short secretory sequence, proendothelin-1 undergoes cleavage by a dibasic pair specific endoprotease—probably furin—to generate the 38 amino-acid peptide, 'big endothelin-1' [6]. Subsequent conversion to the mature, biologically active peptide, endothelin-1, occurs through the action of endothelin converting enzymes (ECE-1 and ECE-2). This family of metalloprotease enzymes is related to neutral endopeptidase-24.11 (NEP) and the Kell protein but unrelated to angiotensin converting enzyme. ECE-1 [14] appears to be the physiologically active ECE and, by alternative gene splicing, it exists in two different isoforms—ECE-1a and ECE-1b—with functionally distinct roles and tissue distributions. ECE-1a appears to be an intracellular enzyme expressed in the Golgi apparatus of cells, such as the endothelial cells, that synthesise endothelin-1. In contrast, responder cells, such as vascular

Figure 3 Factors that alter endothelin-1 (ET-1) synthesis and the pathway for endothelin-1 generation: See text for details. IL-1 = interleukin-1; TGF β = transforming growth factor β ; LDL = low-density lipoprotein; ANP, BNP, CNP = atrial, brain, and c-type natriuretic peptides. (From an original design from Professor P. Vanhoutte).

smooth muscle cells, express extracellular ECE-1b that can convert extracellular big endothelin-1 to mature endothelin-1 [15]. ECE-1 and ECE-2 are both inhibited by phosphoramidon, a combined ECE/NEP inhibitor but not by the selective NEP inhibitor, thiorphan, or by the ACE inhibitor, captopril. Both enzymes are selective for big endothelin-1, raising the possibility that other ECEs with selectivity for big endothelin-2 and -3 will be identified. Through prevention of the generation of the endothelins, ECE-1 must be a potential target for drug treatment.

The gene encoding endothelin-1 can be detected in a wide variety of tissues, including the endothelial and smooth muscle cells of blood vessels, heart, lung, brain, kidney, pancreas and spleen. The gene encoding endothelin-2 may also be found in the vascular endothelium, as well as the smooth muscle of the large and small intestine, myocardium, stomach, kidney, placenta and uterus. Endothelin-3 expression predominates in the brain but is also found in the lung, gastro-intestinal tract and kidney. Big endothelin-1, endothelin-1 and endothelin-3 are present in plasma at picomolar concentrations that are probably insufficient to exert a direct influence on vascular tone. Endothelin-1 is generally thought to be a paracrine and autocrine mediator rather than an endocrine hormone. Indeed, endothelin-1 is largely secreted abluminally by endothelial cells towards the adjacent vascular smooth muscle

[16], so that concentrations are likely to be substantially higher at the interface between endothelial and vascular smooth muscle cells than in blood, consistent with a primarily local action. In addition, the half-life of endothelin-1 in blood is short, at less than 5 min, with clearance predominantly via receptor binding and metabolism in the lungs and kidneys [17].

Endothelin receptors

The endothelins act on two receptor subtypes, ET_A and ET_B, characterized on the basis of their pharmacology (Table 1). Endothelin-1 has a similar binding affinity for ET_A and ET_B receptors—in the nanomolar range—but has a much higher binding affinity for the ET_A receptor than endothelin-3. In contrast, endothelin-1 and endothelin-3 have equal affinity for the ET_B receptor. Understanding of the function of endothelin receptors has been aided by the use of specific pharmacological agonists and antagonists. Endothelin-3 and sarafotoxin S6c are respectively ~2000 fold and ~30 000 fold selective as agonists at the ET_B receptor and BQ-123 and BQ-788 are selective antagonists respectively at ET_A and ET_B receptors. The human ET_A and ET_B receptors have been cloned and exhibit ~60% homology. ET_A receptor mRNA can be detected in many tissues, with the highest expression in aorta, heart and

Table 1 Endothelin receptor characterization.

	Endothelin receptors	
	ET _A	ET _B
Agonist potency	ET-1 > ET-2 >> ET-3	ET-1 = ET-2 = ET-3
Tissue	Aorta	Coronary artery Endothelium
Action	Constriction	Constriction Dilatation
Selective agonists	None	ET-3 (600-fold specific) Sarafotoxin S6c (30 000-fold specific)
Antagonists	BQ-123	BQ-788
	Non-selective bosentan TAK-044	

kidney. The ET_A receptor predominates on vascular smooth muscle cells and is responsible for causing vasoconstriction in both large and small blood vessels [18]. It is also the major receptor subtype in the heart [19]. In contrast, ET_A mRNA cannot be detected in the liver or endothelial cells [20]. The ET_B receptor can be detected in endothelial and vascular smooth muscle cells and is predominantly found in brain, lung, kidney and aorta [21]. The ET_B receptor on endothelial cells modulates vasoconstriction in response to endothelin-1 through the production of vasodilator substances including prostacyclin and nitric oxide. It is now widely recognised that the ET_B receptors on vascular smooth muscle cells can mediate vasoconstriction, particularly in small resistance vessels and veins. The significance of the vasoconstrictor ET_B receptor will be discussed later. There has been a recent tendency to sub-classify the ET_B receptor on the basis of responses to selective agonists and antagonists [22] but this currently cannot be justified on a molecular basis.

The ET_A and ET_B receptors are classical heptahelical rhodopsin-like G-protein coupled receptors that activate phospholipase C leading to hydrolysis of phosphatidyl inositol and generation of cytosolic inositol trisphosphate and membrane bound diacylglycerol [9]. Inositol trisphosphate causes an early rapid rise in $[Ca^{2+}]_i$ through its release from intracellular stores. A more sustained rise of intracellular calcium occurs through opening of membrane Ca^{2+} channels. Diacylglycerol activates protein kinase C, increasing sensitivity of the contractile apparatus to Ca^{2+} , activates nuclear signalling mechanisms—with possible effects on long term regulation of cellular function—and causes a rise in the intracellular pH through an effect on the sodium-hydrogen ion exchange membrane pump. Endothelin-1 may also interact with the ATP-sensitive potassium channel, so contributing to the rise in $[Ca^{2+}]_i$. In addition, it may activate phospholipase A₂, increasing production of arachidonic acid, and hence of prostacyclin (PGI₂) and thromboxane A₂ [10].

The endothelins bind tightly to their receptors in a 'pseudo-irreversible' manner and the endothelin-receptor complex is rapidly internalized. Slow dissociation from their receptors may account for prolonged actions of the endothelins. Endothelin receptor expression is itself regulated by exposure to endothelin-1 so that agents that enhance endothelin-1 production, such as angiotensin II and certain growth factors, can cause endothelin receptor downregulation [23]. In contrast, endothelin receptor number can be

upregulated; for instance, by ischaemia [24], cyclosporin [25] and interleukin-1 β [26].

Developmental biology

Gene defects affecting the endothelin system have been described for both animals and man. Gene knockouts for the preproendothelin-1 gene in mice cause lethal abnormalities affecting the development of craniofacial, cardiovascular and pharyngeal pouch structures [27, 28]. Interestingly, these abnormalities have also been found in teratogenicity studies during the pre-clinical development of a range of ET_A receptor antagonists indicating, at least during development, that endothelin-1 may be the natural ligand for the ET_A receptor and that endothelin-1 has an important role in the development of the pharyngeal arches, heart and great vessels. Indeed, it has been suggested that endothelin-1/ET_A receptor anomalies may contribute to the Pierre-Robin and Treacher-Collins syndromes [29]. One anomalous finding in these studies was the development of raised blood pressure in the endothelin-1 knockout mice. However, evidence is emerging that the elevation of blood pressure is related to sympatho-adrenergic overactivity caused by the severe hypoxia that is a consequence of the facial/pharyngeal anomalies [29].

Both endothelin-3 and ET_B receptor mutations lead to the formation of aganglionic megacolon and coat pigmentation anomalies in animals [30]. In this case, genetic abnormalities of either the preproendothelin-3 gene or the ET_B receptor have been documented in congenital neurocristopathies associated clinically with Hirschsprung's disease [31] and the Waardenberg-Shah syndrome [32]. Hence, the interaction of endothelin-3 with the ET_B receptor appears to be important for the development of cells within the neural crest.

Cardiovascular pharmacology

Bolus administration of endothelin-1 is known to cause a marked pressor effect lasting for more than 60 min [6] in contrast to the brief effects of all other endogenous vasoconstrictor substances. Despite rapid clearance of the peptide from blood, this sustained pressor effect has been confirmed from studies in man [33, 34], and is mediated predominantly through an increase in peripheral vascular resistance. The pressor effect tends to reduce cardiac output [35], probably through a baroreceptor mediated decrease in

heart rate, although an increase in afterload may possibly contribute. Coronary vasoconstriction is recognised to occur in humans who are bitten by the burrowing asp, *Atractaspis engaddensis*, the venom of which contains sarafotoxins [36] and the coronary vasoconstrictor effect of the endothelins has more recently been confirmed in healthy subjects [34]. It is also known that after bolus administration in animals, endothelin-1 causes transient hypotension associated with systemic vasodilatation through stimulation of the endothelial ET_B receptor. However, such studies require high doses of endothelin-1 and, because endothelin-1 preferentially causes vasoconstriction in the renal, cardiac and cerebral circulations, such studies should clearly be avoided in man.

One way to address directly the potential vasoconstrictor and dilator effects of the endothelins *in vivo* in man is to combine bilateral forearm blood flow measurements with unilateral brachial artery infusion of vasoactive drugs at subsystemic, locally active doses. Because forearm muscle blood flow is only $\sim 50 \text{ ml min}^{-1}$ compared with a cardiac output of $\sim 5000 \text{ ml min}^{-1}$ substantial local effects can be achieved without a systemic action. By avoiding confounding effects on organs such as the brain, kidney and heart, as well as potential influences on neurohumoral reflexes, vascular responses can be attributed to a direct effect of the drug, providing a powerful, reproducible and safe method of directly assessing vascular responses *in vivo* [37, 38]. Importantly, the responses obtained are also broadly predictive of those seen in the systemic and coronary circulation [38].

Continuous infusion of endothelin-1 into the brachial artery causes a slowly developing dose-dependent reduction in forearm blood flow, with vasoconstriction sustained for more than 2 h after halting the infusion [8]. When given via the brachial artery, low doses of the ET_B selective agonists, endothelin-3 and sarafotoxin S6c, also produce vasoconstriction in human resistance vessels *in vivo*, consistent with vascular ET_B receptors mediating at least part of the functional response to endothelin-1 in these vessels [39]. In human blood vessels *in vitro*, threshold concentrations of endothelin-1 potentiate contractions to noradrenaline [40]. However, a peripheral interaction of endothelin-1 with the sympathetic nervous system has not been demonstrated in forearm resistance [41] or cutaneous capacitance vessels [42] of healthy subjects *in vivo*. Endothelin-1 and endothelin-3 [39], and sarafotoxin S6c (unpublished observations), can produce transient forearm vasodilatation, the dilator response to endothelin-3 and sarafotoxin S6c being greater and more prolonged than that to endothelin-1 consistent with involvement of the endothelial ET_B receptor. However, vasodilatation to the endothelins occurs only at high doses on bolus administration, suggesting that this is not a physiological response [39]. In human hand veins [43], vasoconstriction is modulated predominantly by stimulated release of dilator prostaglandins.

Endothelin-1 and sarafotoxin S6c both cause sustained constriction of human dorsal hand veins *in vivo* [8, 39], suggesting that both vascular ET_A and ET_B receptors can contribute to venoconstriction to endothelin-1 in humans. Venospasm *in vivo* is blocked more effectively by the K_{ATP} channel opener, cromakalim, than the Ca^{2+} channel antagonist, nicardipine or by hydralazine [44], suggesting

that endothelin-1 responses in human veins depend only in part on Ca^{2+} entry through dihydropyridine-sensitive Ca^{2+} channels. In addition, the greater efficacy of K_{ATP} channel opening agents is consistent with endothelin-1 acting to close K_{ATP} channels, causing plasma membrane depolarisation and vasoconstriction by mechanisms additional to opening of voltage-operated Ca^{2+} channels.

Cardiovascular physiology

Although studies with agonists are of considerable interest, when a mediator is not a classical circulating hormone the results can be frankly misleading and studies with antagonists are likely to be considerably more informative.

The first endothelin 'antagonist' described was the ECE inhibitor, phosphoramidon [45], and we decided to begin our investigation in the forearm. However, to determine a dose that would inhibit ECE we first had to examine responses to big endothelin-1. Brachial artery administration of big endothelin-1 caused a dose-dependent forearm vasoconstriction that could be blocked completely by phosphoramidon (30 nmol min^{-1}), suggesting that the effects of the precursor are mediated through conversion to the mature peptide by ECE [46]. The blockade of constriction to big endothelin-1 by phosphoramidon is unlikely to have been due to inhibition of endothelin receptor binding, because vasoconstriction to endothelin-1 was unaffected by phosphoramidon and because conversion to endothelin-1 and its C-terminal fragment was confirmed in plasma samples taken from the veins draining the infused forearm [47]. Also, because circulating blood exhibits little ECE activity [48], conversion of big endothelin-1 in the forearm presumably occurs via vascular, probably endothelial, ECE situated within the forearm resistance vessels. The difference in potency between big endothelin-1 and endothelin-1, and the ratio of C-terminal fragment to big endothelin-1 in venous blood, both indicated that local ECE converts about 10% of lumenally presented big ET-1 to ET-1, consistent with $\sim 10\%$ conversion of exogenous big ET-1 by cells expressing the ECE-1 gene [14]. Big endothelin-1 does not cause venospasm in hand veins [49], even though these vessels respond to endothelin-1 [8], suggesting that ECE activity may not be present in all vessel types.

Administration of phosphoramidon (30 nmol min^{-1}) alone [46] resulted in a slowly progressive vasodilatation (Figure 4), consistent with a role for endothelin-1 in maintenance of basal vascular tone. Although phosphoramidon is an imperfect tool, because it also acts as an inhibitor of NEP, this latter action is unlikely to explain the vasodilatation because potent and selective inhibitors of NEP cause slowly progressive forearm vasoconstriction [46] (Figure 4). This effect of NEP inhibitors is likely to be caused by accumulation of a vasoconstrictor agent which may be endothelin-1, because it is a substrate for metabolism by NEP and the vasoconstriction is not blocked by an ACE inhibitor. This observation may also account for the increase in plasma endothelin by NEP inhibitors in clinical trials [50] and their failure to lower blood pressure in hypertensive subjects.

Confirmation that endogenous endothelin-1 generation

Figure 4 Forearm vasoconstriction to brachial artery infusion of endothelin-1 (5 pmol min^{-1} ; ●) is abolished by the co-infusion of BQ-123 ($100 \text{ nmol min}^{-1}$; ○). Infusion of the ET_A antagonist BQ-123 ($100 \text{ nmol min}^{-1}$; □) or the ECE inhibitor phosphoramidon (30 nmol min^{-1} ; ●) alone produce progressive forearm vasodilatation whereas the NEP inhibitor thiorphan (30 nmol min^{-1} ; ○) causes progressive vasoconstriction. Adapted from Haynes and Webb [46], with kind permission of the *Lancet*. See text for details.

contributes to the maintenance of basal tone in forearm resistance vessels of healthy human subjects came from studies with the cyclic pentapeptide ET_A receptor selective antagonist, BQ-123 [51], and the cyclic hexapeptide combined $\text{ET}_{A/B}$ antagonist TAK-044 [52], given via the brachial artery. Both agents caused a progressive vasodilatation of the forearm vessels [46, 53]. The substantial effect of BQ-123 (Figure 4) suggests that vasoconstriction to endothelin-1 is mediated mainly through the ET_A receptor on vascular smooth muscle. Subsequent studies have confirmed this effect of BQ-123, and shown that it can be achieved with lower doses and when given for a shorter period [54]. Together, the L-arginine/nitric oxide system, the sympathetic nervous system and the endothelin system are currently the only mediator systems known to maintain basal vascular tone. It now remains to be shown how the endothelin system is regulated, though this is likely to be predominantly at a local level.

The greater effect of BQ-123 than a substantial local dose of TAK-044 would be consistent with a mainly dilator role for the ET_B receptor. Further and more direct support for this view comes from recent arterial experiments with an ET_B selective inhibitor, BQ-788 [55]. This agent causes progressive forearm vasoconstriction in healthy subjects [56], consistent with greater functional importance of the dilator than the constrictor ET_B receptor under physiological circumstances. On this basis, a proportion of the dilator response to BQ-123 is likely to be mediated by nitric oxide and prostacyclin acting through the unopposed endothelial ET_B receptor. It might also be anticipated that this

component of the response might be reduced in conditions associated with endothelial dysfunction (see later). Interestingly, vasoconstriction appears to be mediated solely by ET_A receptors in the human skin microcirculation, again on the basis of studies with antagonists [57].

Responses of the forearm resistance vessels to drugs given into the brachial artery are usually predictive of the responses in the major resistance beds that serve to regulate blood pressure [38]. Indeed, for the L-arginine-nitric oxide system, the substantial—and at the time unexpected—reduction of forearm blood flow associated with administration of the nitric oxide synthase inhibitor, L- N^G -monomethyl-arginine (L-NMMA) [58], predicted the substantial increase in peripheral vascular resistance that would be associated with systemic administration [59]. Similar predictions, though for an opposite effect, were made for systemic endothelin receptor antagonism based on the data with BQ-123. These predictions were confirmed recently in healthy subjects [53] with another peptide drug, TAK-044, which is currently in clinical development.

TAK-044, which functionally inhibits both ET_A and ET_B mediated responses [52, 60], was given to healthy subjects at a range of doses from 10–1000 mg systemically and was generally well tolerated [53]. A 15 min intravenous infusion of TAK-044 at the highest dose of 1000 mg reduced systolic blood pressure by $\sim 4\%$, diastolic blood pressure by $\sim 18\%$, and systemic vascular resistance by $\sim 26\%$ over a 24 h period (Figure 5), suggesting that a major part of the effect of TAK-044 is mediated in the resistance vessels. At this dose, but not consistently at lower doses, the major haemodynamic

Figure 5 Graph showing time course of the effects of the highest dose of TAK-044 (1000 mg) on mean arterial pressure (MAP), heart rate (HR), stroke index (SI), cardiac index (CI), and total peripheral resistance index (TPRI). TAK-044 significantly decreased mean arterial pressure ($P < 0.001$) and total peripheral resistance ($P < 0.001$) and increased heart rate ($P < 0.001$), stroke index ($P = 0.034$) and cardiac index ($P < 0.001$); these effects were maximal at 4 h and sustained for at least 12 h. Data shown represent placebo-corrected changes from predose (change from predose [active] change from predose [placebo]). (AU indicates arbitrary units). Reproduced from Haynes *et al.* [53], with kind permission of the *American Heart Association*.

effect on systemic vascular resistance was accompanied by a compensatory increase in cardiac output and heart rate. The substantial and long lasting effect of TAK-044 on systemic vascular resistance is even more remarkable considering the short half life of this peptide in the circulation and confirms that endogenous endothelin generation is critical for cardiovascular homeostasis and control of blood pressure, as originally proposed by Yanagisawa [6].

Systemically administered TAK-044 also abolished the vasoconstriction to endothelin-1 infused via the brachial artery for up to 3 h [53] but inhibited responses only partially at 8 and 12 h (unpublished data). Such studies can confirm the efficacy of endothelin receptor antagonists and determine the duration of their action. This appears to be especially important for this class of drug, where standard

pharmacokinetic parameters do not always predict the pharmacodynamic activity achieved.

Cardiovascular pathophysiology

There are a number of mechanisms whereby endothelin-1 may be involved in cardiovascular disease, for instance: reduced production in congenital cardiac anomalies; enhanced production in congestive heart disease; reduced receptor number or affinity in Hirschsprung's disease; enhanced receptor number or affinity with cyclosporin treatment; reduced peptide clearance in chronic renal failure; and an unopposed action with endothelial dysfunction affecting the L-arginine/nitric oxide systems, which may be a factor in Raynaud's disease. Given the potentially beneficial

effects of nitric oxide to cause vasodilatation, and inhibit platelet aggregation and vascular growth, and the potentially adverse effects of endothelin-1 to promote vasoconstriction and vascular growth it is of considerable interest that many of the conditions associated with endothelial dysfunction causing reduced nitric oxide production, including atherosclerosis [61, 62], are further compounded by increased production of endothelin-1. Indeed, it is clear that these systems do not function independently. Endothelin-1 generation is enhanced by a range of other constrictor and growth promoting substances and inhibited by dilators including nitric oxide (Figure 3). Conversely, endothelin-1 promotes the production of nitric oxide but may also account for some of the vasoconstriction that accompanies its inhibition by L-NMMA and clinically for the development of tolerance to exogenous nitrate administration [63].

As well as having direct effects on vascular tone, endothelin-1 may enhance vascular tone indirectly: by augmenting vasoconstriction to other agents, such as angiotensin II, noradrenaline, serotonin; by enhancing central and peripheral sympathetic function; and by activating the renin-angiotensin system. Endothelin-1 is also a co-mitogen, enhancing cell division and proliferation, gene expression, protein synthesis and, ultimately, promoting hypertrophy of vascular smooth muscle, as well as cardiac myocytes and fibroblasts [64, 65]. Thus, endothelin-1 may serve to amplify vasoconstriction through the development of vascular hypertrophy.

There is a growing literature [10, 11] in support of a role for endothelin-1 in the pathophysiology of a wide range of cardiovascular diseases. These include ischaemic heart disease and atherosclerosis, as well as conditions associated with either sustained vasoconstriction—including hypertension, chronic heart failure, chronic renal failure, primary pulmonary hypertension—or with intermittent vasospasm—including Raynaud's disease, subarachnoid haemorrhage and acute renal failure. Hereafter, the review will focus on the considerable body of evidence implicating the endothelin system in chronic heart failure and suggesting that it may be a suitable target for therapeutic intervention.

Chronic cardiac failure

Chronic heart failure (CHF) is a common, disabling condition that causes substantial morbidity and mortality, and is a major consumer of health service resources [66]. This complex condition is associated with stimulation of compensatory neurohumoral reflexes, including effects on the renin-angiotensin and sympathetic nervous systems, that serve to maintain perfusion pressure but also act to increase peripheral vascular resistance, renal sodium reabsorption and cardiac workload. This leads to a 'vicious circle' of declining cardiac function and provides a rationale for the current mainstay of treatment, which is vasodilator therapy with ACE inhibitors. Although current treatment regimens are undoubtedly successful, CHF still carries a substantial morbidity and mortality [66] and there is room for additional therapeutic manoeuvres.

The now well documented reduction in mortality with ACE inhibitors in patients with CHF and left ventricular dysfunction after myocardial infarction [66] was predicted

from animal models [67]. A recent report that the ET_A receptor antagonist, BQ-123, substantially improved 12-week survival from 43 to 85% in a coronary occlusion model of CHF [68] as well as haemodynamic function and cardiac remodelling is, therefore, very promising for the clinical developments in this area. Also, interestingly, raised plasma endothelin concentrations appear to be an extremely powerful predictor of 1 year mortality after acute myocardial infarction [69].

Neurohumoral activation and tissue hypoxia should increase endothelin-1 production, and the actions of endothelin-1—vasoconstriction, co-mitogenesis, leading to cardiac and vascular hypertrophy, enhancement of renin-angiotensin and sympathetic nervous system activity, and promotion of renal vasoconstriction and sodium retention—are all consistent with the circulatory abnormalities found in this condition. Indeed, plasma endothelin concentrations are elevated in CHF, mainly through an increase in plasma big endothelin-1 [70], consistent with increased synthesis, rather than decreased clearance, of endothelin-1. Plasma immunoreactive endothelin correlates with the degree of haemodynamic [71] and functional impairment [72] in CHF, is associated with a worse prognosis—irrespective of the cause of the cardiac failure—and predicts mortality or the need for cardiac transplantation [73]. Currently, measurement of plasma big endothelin-1 concentration is the best available predictor of outcome in CHF [74]. Interestingly, changes in plasma immunoreactive endothelin reflect the clinical response to the β -adrenoceptor blocker carvedilol in patients with CHF [75], although it is not clear whether the drug is producing its benefits through direct inhibition of the endothelin system or by an effect on cardiac performance.

In the first clinical trial of an endothelin antagonist in CHF [12], in patients withdrawn from ACE inhibitor treatment, acute intravenous administration of the combined ET_{A/B} antagonist, bosentan, increased cardiac output and reduced systemic and pulmonary vascular resistance without inducing reflex tachycardia or increasing plasma concentrations of angiotensin II or noradrenaline (Figure 6). These beneficial haemodynamic effects of inhibition of the endothelin system are similar to those associated with ACE inhibition, and beg the question whether they would add to the effects of optimal treatment with an ACE inhibitor [76]. Studies with local brachial artery administration of the ECE inhibitor, phosphoramidon, and the ET_A receptor antagonist, BQ-123, have addressed this issue in patients with CHF [77]. Even though these patients were maintained on ACE inhibitors, both agents caused substantial vasodilatation of the forearm resistance vessels, predicting that endothelin receptor antagonists might have additional value in the treatment of CHF. Indeed, from recent studies, it does appear that the beneficial haemodynamic effects of bosentan occur in the presence of ACE inhibitors and are sustained on chronic oral treatment (W. Kiowski; personal communication).

In local infusion studies, the vasoconstriction to endothelin-1 was reduced in CHF patients compared with matched control subjects in both resistance vessels [77] and hand veins [78] consistent with increased endothelin-1 generation. In contrast, vasoconstriction to the selective ET_B receptor agonist, sarafotoxin S6c, was enhanced. These

Figure 6 Changes of cardiac index, stroke volume, systemic vascular resistance and pulmonary vascular resistance (a) and of arterial, pulmonary artery, pulmonary artery wedged and right atrial pressures (b) in patients with severe congestive heart failure after intravenous placebo (unshaded columns) or bosentan (shaded columns). Bosentan 100 mg was administered intravenously at 0 min and a further 200 mg was given at 60 min. All eight parameters were significantly improved by acute administration of bosentan ($P < 0.05$) without change in heart rate. Adapted and reproduced from Kiowski *et al.* [12], with kind permission of the *Lancet*.

observations are also seen in the coronary vessels in experimental CHF [79] and ET_B receptors are also upregulated in human CHF [80], consistent with widespread upregulation of the smooth muscle, and perhaps endothelial, ET_B receptor in this condition. Enhanced constriction to sarafotoxin S6c may, at least in part, be due to endothelial dysfunction affecting responses mediated through the endothelial ET_B receptor and suggests that constrictor ET_B may be of greater importance in some diseases than they are under physiological circumstances. Nevertheless, in patients with CHF, the response to arterial administration of the selective ET_B antagonist, BQ-788, is vasoconstriction, suggesting that the dilator response predominates and that selective ET_A antagonists might, therefore, offer some advantages in this condition.

Unresolved issues

A number of major issues concerning endothelin antagonists remain unresolved. The first and most important of these is the choice of the appropriate therapeutic target. On purely scientific grounds there would certainly be sufficient justification for clinical investigation of endothelin antagon-

ists in essential hypertension, congestive heart failure, primary pulmonary hypertension, subarachnoid haemorrhage, stroke and acute ischaemic renal failure. However, there are a number of other issues for pharmaceutical companies to consider including the existence of currently effective treatment (essential hypertension), the lack of a sufficiently predictive model for the disease (angioplasty restenosis) and concerns over whether the market size is sufficiently large to justify the development (subarachnoid haemorrhage and primary pulmonary hypertension). There is obviously also a need to keep the overall budget for such compounds within reasonable bounds so companies need to be selective in their research programmes. Nevertheless, it can be expected that the role of endothelin antagonists will be explored in clinical studies in a number of these candidate diseases and it is likely—as, for instance, with the capacity of ACE inhibitors to delay the progression of renal failure—that some of the potential uses of endothelin antagonists cannot easily be anticipated at this stage. For instance, the potential anti-mitogenic action of endothelin antagonists may be critical in heart failure [68] and perhaps also in conditions like essential and pulmonary hypertension, and may even be relevant for cancer therapy [81].

Although the first effective 'endothelin antagonist' was an ECE inhibitor there appear to have been few recent developments in this area. All of the four or more endothelin antagonists in early clinical development, as well as at least 15 more in preclinical development [11], are endothelin receptor blockers. Most of these are orally active, although TAK-044 is a peptide and is therefore being developed for indications requiring relatively brief administration. Some of these agents are combined ET_{A/B} antagonists, whereas others are selective ET_A antagonists. There are currently no selective ET_B antagonists that are clearly intended for clinical development although it might be argued that they would produce an organ-selective effect in pulmonary hypertension, avoiding systemic hypotension, given that hypoxic pulmonary vasoconstriction appears to be primarily mediated by ET_B receptors [82] whereas responses to endothelin-1 in the peripheral circulation appear to be determined primarily by effects on the ET_A receptor.

The more general issue of whether selective ET_A or combined ET_{A/B} receptor antagonists will have greater utility has certainly not yet been resolved. Our current knowledge, from studies in healthy people and those with heart failure is that the major target must be the ET_A receptor. Inhibition of the ET_B receptor leads to peripheral vasoconstriction and, unlike inhibition at the ET_A receptor, causes substantial elevation of plasma concentrations of endothelin-1 [12, 53], probably by effects on clearance or displacement from ET_B receptors [83, 84]. However, the function of ET_B receptor may be more critical in some diseases or in, for instance, the cardiac and renal vascular beds, and the role of ET_B receptors in overall human cardiovascular control and pathophysiology has yet to be determined.

Summary

Endothelin-1 is an endothelium-derived vasoconstrictor and co-mitogenic agent which acts as a local paracrine and autocrine mediator, and is the most potent and sustained vasoconstrictor and pressor substance yet identified. On the basis of studies in healthy man, endothelin-1 is now known to play an important physiological role in maintaining peripheral vascular tone and blood pressure. Endothelin-1 also has actions which might influence the function of the heart, kidney and nervous system. However, their physiological importance remains to be determined.

Abnormalities of the endothelin system are now recognised to occur in a range of diseases associated with vasoconstriction, vasospasm and vascular hypertrophy and it appears that endothelin-1 may be causal, or at least contributory, in some of these pathophysiological processes. The use of endothelin receptor antagonists in experimental models of cardiovascular disease and in human clinical pharmacology studies has indicated a number of conditions—including hypertension, heart failure, acute renal failure, subarachnoid haemorrhage, and pulmonary hypertension—in which further clinical studies would be worthwhile. A number of peptide and orally-active non-peptide endothelin receptor antagonists are now under clinical investigation and further studies are now required in specific diseases to determine whether

selective ET_A or combined ET_{A/B} receptor antagonists would be more effective.

The discovery of endothelin-1, and the design of endothelin antagonists, has been among the most promising developments in cardiovascular medicine since the launch of ACE inhibitors 15 years ago. Major clinical trials are now needed to confirm the predicted benefits for endothelin antagonists in patients with cardiovascular disease.

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