

## Review article

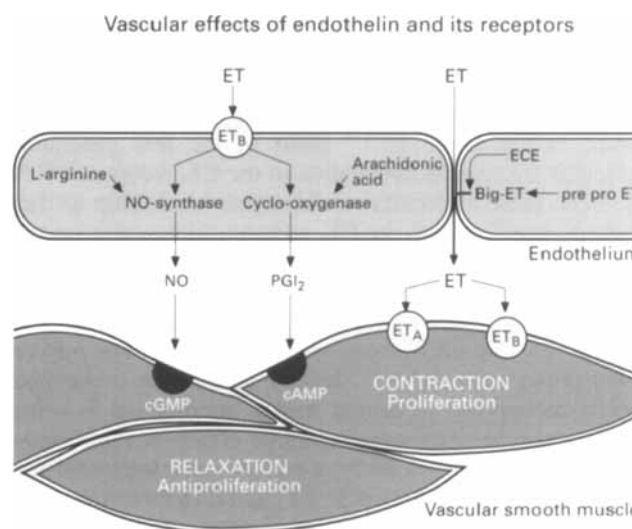
# Do we need endothelin antagonists?

Thomas F Lüscher

The arsenal of therapeutic agents available in clinical practice has expanded dramatically in the last 100 years. In cardiovascular pharmacotherapy, digoxin and nitrates were the first agents available.<sup>1,2</sup> In the last decades, diuretics, reserpine,  $\beta$  blockers,<sup>3</sup> calcium antagonists,<sup>4</sup> and most recently angiotensin converting enzyme inhibitors<sup>5</sup> have been added to the armamentarium of cardiovascular care. The advent of a new class of drugs is a rare event in most areas of medicine and usually occurs once in a decade or even less.

Drugs may either be introduced solely on the basis of circumstantial evidence that they work or they may be derived from the discovery of a new regulatory system. An example of the former is the introduction of nitrates by Thomas Lauder Brunton in the last century.<sup>2</sup> Indeed, Dr Brunton had no idea of the mechanism of action of the drugs he was using, nor did he understand the disease he was treating. Yet his therapeutic approach is still common clinical practice.<sup>6</sup> On the other hand,  $\beta$  blockers and particularly angiotensin converting enzyme inhibitors have been derived from the discovery of new regulatory mechanisms in the cardiovascular system. Similarly, endothelin antagonists have now become available shortly after the discovery of the potent 21 amino acid peptide endothelin by Yanagisawa and coworkers in 1988<sup>7</sup> and the cloning of its receptors in 1990.<sup>8-10</sup>

Endothelins are a family of peptides with potent biological effects.<sup>7-16</sup> Endothelin-1, -2, and -3 are formed from precursor molecules (big endothelin) via the activity of a putative endothelin converting enzyme (ECE; figure). Endothelin-1 appears to be the primary product of endothelial cells. In vitro and in vivo, endothelin is a very potent vasoconstrictor<sup>7,11-17</sup> but under certain conditions, however, it also causes transient vasodilatation.<sup>7,12,18</sup> In addition, endothelin potentiates the effects of other vasoconstrictor hormones,<sup>19-21</sup> stimulates migration and proliferation of vascular smooth muscle,<sup>22-24</sup> and exerts renal and endocrine effects.<sup>25</sup> Two endothelin receptors have been cloned, the  $ET_A$  receptor which preferentially binds endothelin-1<sup>8</sup> and the  $ET_B$  receptor which has an equal affinity for all isoforms of endothelins.<sup>9</sup> In addition, a putative  $ET_C$  receptor with preferential binding of endothelin-3 has also been proposed,<sup>26</sup> although its existence remains controversial.<sup>27</sup> The vasoconstrictor effects of endothelin in vascular smooth muscle are mediated by  $ET_A$  receptors and in certain blood vessels also by an  $ET_B$  receptor.<sup>28-30</sup> The vasodilator actions of endothelins, which are particularly seen with intraluminal application of a bolus of the peptide and in small and



*Vascular effects of endothelin (ET) and its receptors:  $ET_A$  and  $ET_B$  receptors are expressed on vascular smooth muscle to mediate contraction and proliferation, while endothelial cells only express  $ET_B$  receptors linked to the formation of nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>). These mediators are responsible for the vasodilatation occurring under these conditions via increases in cyclic GMP and cyclic AMP, respectively. ECE = endothelin converting enzyme; circles = receptors.*

resistance arteries,<sup>7,12,18</sup> is mediated by an  $ET_B$  receptor on endothelial cells linked to the formation of vasodilator substances such as nitric oxide and prostacyclin (figure).

Although endothelin has impressive biological effects within the cardiovascular system on vascular tone and growth as well as on renal function, its physiological role still remains controversial. This is mainly due to the lack of specific inhibitors interfering with the effects of endogenously produced endothelin. Indeed the fact that circulating levels of endothelins are very low under normal conditions suggests that the peptide acts as a local vascular regulatory system rather than as a circulating hormone. This also makes it unlikely that the vast number of pharmacological studies published<sup>see 11</sup> with infusion or application of high concentrations of endothelin do indeed have physiological relevance. Although the question raised in this review appears to be aiming at therapeutic implications of the newly developed endothelin antagonists, an equally important aspect of such molecules may be the fact that they will serve as specific tools to study the physiological importance of

**Table I** Endothelin antagonists.

Name	Supplier	Structure	Effect
BE-18257A	Banyu	Cyclo(-D-Glu-L-Ala-D-Val-L-Leu-D-Trp-)	ET <sub>A</sub>
BE-18257B	Banyu	Cyclo(-D-Glu-L-Ala-D-allo-Ile-L-Leu-D-Trp-)	ET <sub>A</sub>
BQ-162	Banyu	Cyclo(-D-Glu-L-Pro-D-Val-L-Leu-D-Trp-)	ET <sub>A</sub>
BQ-123	Banyu	Cyclo(-D-Asp-L-Pro-D-Val-L-Leu-D-Trp-)	ET <sub>A</sub>
BQ-153	Banyu	Cyclo(-D-Sal*-L-Pro-D-Val-L-Leu-D-Trp-)	ET <sub>A</sub>
FR 139317	Fujisawa	(R)2-[(R)-2-[(S)-2-[[1-hexahydro-1H-azepinyl]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1H-indolyl)propionyl]amino-3-(2-pyridyl)propionic acid	ET <sub>A</sub> ET <sub>A</sub> /ET <sub>B</sub>
RO 46-2005	Hoffmann-La Roche		ET <sub>A</sub> /ET <sub>B</sub>
SB 209670	SmithKline Beecham		ET <sub>A</sub> /ET <sub>B</sub>
PD142893	Parke-Davis		ET <sub>A</sub> /ET <sub>B</sub>
PD145065	Parke-Davis		ET <sub>A</sub> /ET <sub>B</sub>
PD147953	Parke-Davis		ET <sub>A</sub> /ET <sub>B</sub>

\* = D-Sulphoalanine

endogenously formed endothelin in the cardiovascular system.

An increasing number of molecules interfering with one or both endothelin receptors have become available for experimental studies (table I).<sup>32-43</sup> Most of the first generation molecules are specific antagonists of the ET<sub>A</sub> receptor.<sup>33-36</sup> At first sight, these molecules would appear ideal drugs as they primarily interfere with the ET<sub>A</sub> receptor on vascular smooth muscle which mediates vasoconstriction, while leaving the ET<sub>B</sub> receptor on endothelial cells untouched (figure). It soon became apparent, however, that in several vascular beds, ET<sub>B</sub> receptors on vascular smooth muscle also contribute importantly to vasoconstriction.<sup>28-30</sup> Indeed, particularly in the renal circulation but also in certain human arteries and in veins, ET<sub>B</sub> receptors mediate vasoconstrictor effects of the peptide. This led to a change in concept and strongly suggested that molecules interfering with both ET<sub>A</sub> and ET<sub>B</sub> receptors might be much more appropriate for further development as therapeutic agents. Several of these compounds are now tested at the experimental level (table I).<sup>44-46</sup> A major drawback of the molecules at this point is the fact that they cannot be used for human *in vivo* studies yet and are usually only poorly available via the oral route. Furthermore, their safety is uncertain.

Do we need endothelin antagonists in clinical medicine and if so, for which diseases? There are a number of disease states associated with increased circulating levels of endothelin (table II).<sup>see 11</sup> Indeed, almost every vascular disease such as atherosclerosis,<sup>47</sup> Takayasu's disease, Raynaud's disease,<sup>48</sup> and others is associated with increased circulating levels of the peptide. Similarly, in migraine,<sup>49</sup> cerebral vasospasm after subarachnoid haemorrhage,<sup>50</sup> and coronary spasm,<sup>51, 52</sup> increased local or circulating levels of the peptide have been reported. There is also good experimental and clinical evidence that endothelin production is stimulated during myocardial ischaemia and infarction<sup>53, 54</sup> and that the

increased production of the peptide may contribute to infarct size and extension.<sup>55</sup> Endothelin may be stimulated under these conditions by thrombin<sup>7, 56</sup> which is abundantly present in clots, as well as by hypoxia.<sup>57</sup> Hypoxia and ischaemia can also externalise endothelin receptors.<sup>58</sup>

Pulmonary hypertension is another clinical syndrome in which endothelin has been implicated,<sup>59</sup> while in systemic arterial hypertension, the published results are very controversial.<sup>see 11, 32</sup> Indeed, while some investigators found normal values in patients with essential hypertension, others reported increased levels, most commonly in small sample studies.<sup>see 11, 60-67</sup> The controversial results in arterial hypertension may be related to the presence or absence of vascular disease and/or renal failure or possibly also reflect a heterogeneity of hypertensive patients with regard to the activation of endothelin production. Increased vascular endothelin levels could certainly mediate an increase in peripheral vascular resistance which is the hallmark of arterial hypertension. True endothelin dependent hypertension has been described in patients with endothelin producing tumours (haemangiopericytomas) in which the contribution of endothelin to blood pressure regulation is obvious.<sup>68</sup> In addition to these cardiovascular disease states, endothelin has been implicated in other clinical conditions such as asthma,<sup>69, 70</sup> while its role in Crohn's disease is controversial.<sup>71, 72</sup>

However, we should not derive our indications only from the fact that circulating endothelin levels are increased in a given disease state. Indeed, as endothelin primarily acts as a local vascular regulator and two thirds of the endothelin produced by endothelial cells is released abluminally rather than lumenally,<sup>73</sup> endothelin may contribute importantly to a cardiovascular disease process even in the presence of normal circulating levels of the peptide. In line with this concept, endothelin antagonists,<sup>74</sup> in particular those interfering with both the ET<sub>A</sub> and ET<sub>B</sub> receptors but also endothelin converting enzyme inhibitors,<sup>75</sup> do lower blood pressure in spontaneous and DOCA salt hypertension in the rat,<sup>74</sup> although circulating levels of endothelin are normal or even low in these models of hypertension.<sup>60</sup> Similarly, local spastic events in certain parts of the cardiovascular system such as the coronary<sup>51, 52</sup> and cerebral circulation<sup>50</sup> may be mediated by an increased endothelin production in the diseased vascular segment, but the amount of endothelin released lumenally may not be sufficient to increase circulating endothelin levels in these patients.

What would be the most appropriate indications for endothelin antagonists in clinical medicine? Obviously this question cannot be addressed appropriately as the data supporting one statement or another are still lacking. However, experimental studies strongly suggest that endothelin

**Table II** Disease states with increased circulating endothelin levels.

I	Vascular disease	Atherosclerosis Takayasu's disease Raynaud's disease
II	Heart disease	Myocardial infarction Coronary spasm Cardiac shock Heart failure
III	Hypertension	Arterial hypertension (?) Pulmonary hypertension
IV	Other diseases	Migraine Subarachnoid haemorrhage Renal failure (acute and chronic) Hepatorenal syndrome

antagonists are able to lower blood pressure. In addition they may have the capacity to reduce the occurrence of cardiovascular complications of the hypertensive process such as stroke.<sup>74</sup>

If endothelin antagonists do lower blood pressure, do we need another antihypertensive agent, as so many are already available? It is obvious that the currently available drugs such as diuretics,  $\beta$  blockers, ACE inhibitors, and calcium antagonists are very potent and well tolerated antihypertensive drugs which are more or less equally effective in lowering blood pressure. Combination therapy with these antihypertensive drugs allows control of the blood pressure in the vast majority of hypertensive patients. Hence from a haemodynamic point of view another antihypertensive agent is not what the cardiovascular community is necessarily waiting for. However, antihypertensive therapy is far from perfect with regard to the prevention of hypertensive complications (for example, stroke, myocardial infarction, and renal failure) and this indeed is the true aim of antihypertensive therapy. Currently, antihypertensive drugs are quite effective in reducing haemorrhagic and ischaemic stroke as well as left ventricular failure in patients with hypertension. However, even treated hypertensive patients do not reach the risk profile of normotensive patients and the effect of antihypertensive drugs on coronary artery disease and renal dysfunction (at least in certain patients) is even less satisfying. Hence there is a place for a new antihypertensive agent capable not only of lowering pressure but also of effectively interfering with vascular dysfunction and in turn preventing vascular morbidity and death. Endothelin may be an important mediator in this context as the peptide is a locally released vasoconstrictor which also has migratory and proliferative properties.<sup>22-24</sup> The fact that the peptide is produced in increased amounts in any form of human vascular disease<sup>47-48</sup> suggests that it is either a marker or an important mediator of vascular damage.

Furthermore, experiments with cyclosporin induced renal dysfunction indicate that endothelin antagonists may have a role in preventing the untoward effects of cyclosporin within the kidney and the vasculature.<sup>76-78</sup> Indeed, infusion of endothelin antagonists can prevent the decrease in renal blood flow induced by cyclosporin A.<sup>79</sup>

In addition, endothelin antagonists may be very promising in preventing and possibly also reversing acute renal failure.<sup>80</sup> In both acute and chronic renal failure, plasma endothelin levels are increased.<sup>65-81</sup> Most interestingly, endothelin antagonists have been shown to prevent or reduce cerebral vasospasm occurring after experimental subarachnoid haemorrhage.<sup>83-85</sup> In addition, at this stage we certainly should keep our eyes open and test this new class of drugs in pulmonary hypertension, migraine, coronary spasm, and evolving myocardial infarction. Indeed, in myocardial infarction the duration of the increase of circulating endothelin has been related to the outcome of the patients<sup>54</sup> and at the experimental levels, endothelin antibodies have been shown to reduce infarct size.<sup>55-86</sup> Non-cardiovascular disease states such as asthma,<sup>69-70</sup> hepatorenal syndrome,<sup>87</sup> and certain forms of ulcer also might be considered.

Thus endothelin antagonists may provide new and potentially effective tools to interfere with vascular disease and its complications in different organs. Given this background, the imminent availability of endothelin antagonists is an exciting prospect both for scientists and clinicians. As scientists and clinical investigators we certainly need endothelin antagonists to teach us more about the (patho)physiology of cardiovascular disease. In the not too

distant future, this will also allow us to answer the question whether as clinicians we truly need this new class of drugs to treat patients.

Received 21 July 1993; accepted 11 August 1993. Time for primary review 21 days.

- 1 Withering W. An account of the foxglove and some of its medical uses with practical remarks on dropsy, and other diseases. In: Willis FA, Keys TE, eds. *Classics in cardiology*. New York: Henry Schuman Inc, 1941: 231.
- 2 Brunton TL. On the use of nitrate amyl in angina pectoris. *Lancet* 1867;ii:97.
- 3 Cruickshank JM, Pritchard BNC. *Beta-blockers in clinical practice*. Edinburgh: Churchill Livingstone, 1987.
- 4 Nayler WG. *Calcium antagonists*. London: Academic Press, 1988.
- 5 Sweet CS, Blaine EH. Angiotensin-converting enzyme and renin inhibitors. In: Antonaccio MJ, ed. *Cardiovascular pharmacology*. New York: Raven Press, 1984:119-54.
- 6 Abrams J. Nitroglycerin and long-acting nitrates. *N Engl J Med* 1980;**302**:1234-7.
- 7 Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;**332**:411-5.
- 8 Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding and endothelin receptor. *Nature* 1990;**348**:730-2.
- 9 Sakurai T, Yanagisawa M, Takawa Z, et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* 1990;**348**:732-5.
- 10 Vane J. Endothelins come home to roost. *Nature* 1990;**348**: 673-5.
- 11 Lüscher TF, Boulanger CM, Dohi Y, Yang Z. Endothelium-derived contracting factors. *Hypertension* 1992;**19**:117-30.
- 12 Dohi Y, Lüscher TF. Endothelin-1 in hypertensive resistance arteries: intraluminal extraluminal dysfunction. *Hypertension* 1991;**18**:543-9.
- 13 Miller WL, Redfield MM, Burnett JC. Integrated cardiac, renal, and endocrine actions of endothelin. *J Clin Invest* 1989;**83**: 317-20.
- 14 Kiowski W, Lüscher TF, Linder L, Bühler FR. Endothelin-1-induced vasoconstriction in humans: reversal by calcium channel blockade but not by nitrovasodilators or endothelium-derived relaxing factor. *Circulation* 1991;**83**:469-75.
- 15 Brain SD, Tippins JR, Williams TJ. Endothelin induces potent microvascular constriction. *Br J Pharmacol* 1988;**95**:1005-7.
- 16 De Nucci G, Thomas R, D'Orleans-Juste P, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 1988;**85**:9797-800.
- 17 Clarke JG, Larkin SW, Benjamin N, et al. Endothelin-1 is a potent long-lasting vasoconstrictor in dog peripheral vasculature in vivo. *J Cardiovasc Pharmacol* 1989;**13**(suppl 5):211-2.
- 18 Wright CE, Fozard JR. Regional vasodilation is a prominent feature of the haemodynamic response to endothelin in anaesthetized, spontaneously hypertensive rats. *Eur J Pharmacol* 1988;**155**:201-3.
- 19 Tabuchi Y, Nakamaru M, Rakugi H, Nagano M, Ogihara X. Endothelin enhances adrenergic vasoconstriction in perfused rat mesenteric arteries. *Biochem Biophys Res Commun* 1989;**159**: 1304-8.
- 20 Yang Z, Richard V, von Segesser L, et al. Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries: a new mechanism of vasospasm? *Circulation* 1990;**82**:188-95.
- 21 Godfraind T, Mennig D, Morel N, Wibo M. Effect of endothelin-1 on calcium channel gating by agonists in vascular smooth muscle. *J Cardiovasc Pharmacol* 1989;**13**(suppl 5): 112-7.
- 22 Hirata Y, Takagi Y, Fukuda Y, Marumo F. Endothelin is a potent mitogen for rat vascular smooth muscle cells. *Atherosclerosis* 1989;**78**:225-8.
- 23 Simonson MS, Wann S, Mené P, et al. Endothelin stimulates phospholipase C,  $\text{Na}^+/\text{H}^+$  exchange, c-fos expression, and mitogenesis in rat mesangial cells. *J Clin Invest* 1989;**83**: 708-12.
- 24 Dubin D, Pratt RE, Cooke JP, Dzau VJ. Endothelin, a potent vasoconstrictor, is a vascular smooth muscle mitogen. *J Vasc Med Biol* 1989;**1**:13-17.

- 25 Miller WL, Redfield MM, Burnett JC. Integrated cardiac, renal, and endocrine actions of endothelin. *J Clin Invest* 1989;**83**: 317–20.
- 26 Emori T, Hirata Y, Marumo F. Specific receptors for endothelin-3 in cultured bovine endothelial cells and its cellular mechanism of action. *FEBS Lett* 1990;**263**:261–4.
- 27 Masaki T, Angus J, Rubanyi GM, *et al.* Endothelin receptor classification. *Pharmacol Rev* (in press).
- 28 Clozel M, Gray GA, Breu V, Löffler BM, Osterwalder R. The endothelin ET<sub>B</sub> receptor mediates both vasodilation and vasoconstriction in vivo. *Biochem Biophys Res Commun* 1992;**186**: 867–73.
- 29 Harrison VJ, Randrianosa A, Schoeffter P. Heterogeneity of endothelin-sarafotoxin receptors mediating contraction of pig coronary artery. *Br J Pharmacol* 1992;**105**:511–3.
- 30 Moreland S, McMullen DM, Delaney CL, Lee VG, Hunt JT. Venous smooth muscle contains vasoconstrictor ET<sub>B</sub>-like receptors. *Biochem Biophys Res Commun* 1992;**184**:100–6.
- 32 Lüscher TF, Seo BG, Bühler FR. Potential role of endothelin in hypertension (Controversy on endothelin in hypertension). *Hypertension* 1993;**21**:752–7.
- 33 Ihara M, Noguchi K, Saeki T, *et al.* Biological profiles of highly potent novel endothelin antagonists selective for the ET<sub>A</sub> receptor. *Life Sci* 1992;**50**:247–55.
- 34 Ihara M, Saeki T, Funabashi, *et al.* Two endothelin receptor subtypes in porcine arteries. *J Cardiovasc Pharmacol* 1991;**17**(suppl 7):S119–21.
- 35 Nakamichi K, Ihara M, Kobayashi M, Saeki T, Ishikawa K, Yano M. Different distribution of endothelin receptor subtypes in pulmonary tissues revealed by the novel selective ligands BQ-123 and [Ala<sup>1</sup>,3,11]ET-1. *Biochem Biophys Res Commun* 1992;**182**: 144–50.
- 36 Hiley CR, Cowley DJ, Pelton JT, Hargreaves AC. BQ-123, cyclo(D-Trp-D-Asp-Pro-D-Val-Leu), is a non-competitive antagonist of the actions of endothelin-1 in SK-N-MC human neuroblastoma cells. *Biochem Biophys Res Commun* 1992;**184**:953–9.
- 37 Webb ML, Dickinson KE, Delaney CL, *et al.* The endothelin receptor antagonist, BQ-123, inhibits angiotensin II-induced contractions in rabbit aorta. *Biochem Biophys Res Commun* 1992;**185**:887–92.
- 38 Ohlstein EH, Arleth A, Bryan H, Elliott JD, Sung CP. The selective endothelin ETA receptor antagonist BQ-123 antagonizes endothelin-1-mediated mitogenesis. *Eur J Pharmacol* 1992;**225**: 347–50.
- 39 Atkinson RA, Pelton JT. Conformational study of cyclo-[D-Trp-D-Asp-Pro-D-Val-Leu], an endothelin-A receptor-selective antagonist. *FEBS Lett* 1992;**296**:1–6.
- 40 Clozel M, Fischli W, Löffler B-M, Breu V. The discovery of R 46-2005, an orally available non-peptide antagonist of ET<sub>A</sub> and ET<sub>B</sub> receptors. Abstract, 3rd International Conference on Endothelin, Houston, 1993.
- 41 Miyata S, Fukami N, Neya M, Takase S, Kiyoto S. WS-7338, new endothelin receptor antagonists isolated from *Streptomyces* sp. NO. 7338: III. Structures of WS-7338 A, B, C and D and total synthesis of WS-7338 B. *J Antibiot (Tokyo)* 1992;**45**:788–91.
- 42 Miyoto S, Hashimoto M, Fujie K, *et al.* SW009 A and B, new endothelin receptor antagonists isolated from *Streptomyces* sp. no. 89009: II. Biological characterization and pharmacological characterization of WS009 A and B. *J Antibiot (Tokyo)* 1992;**45**: 1041–6.
- 43 Meyer P, Flammer J, Lüscher TF. Endothelium-dependent regulation of the ophthalmic microcirculation in the perfused porcine eye: Role of nitric oxide and endothelins. *Invest Ophthalmol Vis Sci* (in press).
- 44 Sun XY, Hedner T, Feng Q, Edvinsson L. Inhibition of endothelin (ET-1) induced pressor responses by the endothelin (ETA) receptor antagonist FR139317 in the pithed rat. *Blood Pressure* 1992;**1**:108–12.
- 45 Nishikibe M, Ikada M, Tsuchida S, *et al.* Antihypertensive effect of a newly synthesized endothelin antagonists, BQ-123, in genetic hypertension models. (Abstract) *J Hypertens* 1992;**10**(suppl 4):P53.
- 46 Bazil MK, Lappe RW, Webb RL. Pharmacologic characterization of an endothelin<sub>A</sub> (ET<sub>A</sub>) receptor antagonist in conscious rats. *J Cardiovasc Pharmacol* 1992;**20**:940–8.
- 47 Lerman A, Edwards BS, Hallett JW, Heublein DM, Sondberg SM, Burnett JC. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med* 1991;**325**:997–1001.
- 48 Zamora MR, O'Brien RF, Rutherford RB, Weil JV. Serum endothelin-1 concentrations and cold provocation in primary Raynaud's phenomenon. *Lancet* 1990;**336**:1144–7.
- 49 Farkkila M, Palo J, Saijonmaa O, Gyhrquist F. Raised plasma endothelin during acute migraine attack. *Cephalalgia* 1992;**12**:383–4.
- 50 Masaoka H, Suzuki R, Hirata Y, Emori T, Marumo F, Hirakawa K. Raised plasma endothelin in aneurysmal subarachnoid haemorrhage. *Lancet* 1989;ii:1402.
- 51 Toyo-oka T, Aizawa T, Suzuki N, *et al.* Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. *Circulation* 1991;**83**:476–83.
- 52 Lüscher TF. Endothelin: key to coronary vasospasm? (Editorial comment). *Circulation* 1991;**83**:701–3.
- 53 Myauchi T, Yanagisawa M, Tomizawa T, *et al.* Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. *Lancet* 1992;ii:53–4.
- 54 Stewart DJ, Kubac G, Costello KB, Cernacek P. Increased plasma endothelin-1 in the early hours of acute myocardial infarction. *J Am Coll Cardiol* 1991;**18**:38–43.
- 55 Watanabe T, Suyuki N, Shimamoto N, Jujino M, Imada A. Endothelin in myocardial infarction. *Nature* 1990;**344**:114–9.
- 56 Boulanger CM, Lüscher TF. Endothelin is released from the porcine aorta: inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990;**85**:587–90.
- 57 Rakugi H, Tabuchi Z, Nakamura M, *et al.* Evidence for endothelin-1 release from resistance vessels of rats in response to hypoxia. *Biochem Biophys Res Commun* 1990;**169**:973–7.
- 58 Liu J, Casley DJ, Nayler WG. Ischaemia causes externalization of endothelin-1 binding sites in rat cardiac membranes. *Biochem Biophys Res Commun* 1989;**164**:1220–5.
- 59 Cernacek P, Stewart DJ. Immunoreactive endothelin in human plasma: Marked elevations in patients in cardiogenic shock. *Biochem Biophys Res Commun* 1989;**161**:562–7.
- 60 Suzuki N, Miyauchi T, Tomobe Y, *et al.* Plasma concentrations of endothelin-1 in spontaneously hypertensive rats and DOCA-salt hypertensive rats. *Biochem Biophys Res Commun* 1990;**167**: 941–7.
- 61 Miyauchi T, Yanagisawa M, Suzuki N, *et al.* Venous plasma concentrations of endothelin in normal and hypertensive subjects. (Abstract) *Circulation* 1989;**80**(suppl II):II-2280.
- 62 Davenport AP, Ashby MJ, Easton P, *et al.* A sensitive radioimmunoassay measuring endothelin-like immunoreactivity in human plasma: comparison of levels in patients with essential hypertension and normotensive control subjects. *Clin Sci* 1990;**78**:261–4.
- 63 Schrader J, Tebbe U, Borries M, *et al.* Plasma Endothelin bei Normalpersonen und Patienten mit nephrologisch-rheumatischen und kardiovaskulären Erkrankungen. *Klin Wochenschr* 1990;**68**:774–9.
- 64 Schiffrin EL, Thibault G. Plasma endothelin in human essential hypertension. *Am J Hypertens* 1991;**4**:303–8.
- 65 Shihiri M, Hirata Y, Ando K, *et al.* Plasma endothelin levels in hypertension and chronic renal failure. *Hypertension* 1990;**15**: 493–6.
- 66 Kohno M, Yasunari K, Murakawa K, *et al.* Plasma immunoreactive endothelin in essential hypertension. *Am J Med* 1990;**88**:614–8.
- 67 Saito Y, Nakao K, Mukoyama M, Imura H. Increased plasma endothelin level in patients with essential hypertension. *N Engl J Med* 1990;**322**:305.
- 68 Yokokawa K, Tahara H, Kohno M, *et al.* Hypertension associated with endothelin-secreting malignant hemangioendothelioma. *Ann Intern Med* 1991;**114**:213–5.
- 69 Mattoli S, Soloperto M, Marini M, Fasoli A. Levels of endothelin in the bronchoalveolar lavage fluid of patients with symptomatic asthma and reversible airflow obstruction. *J Allergy Clin Immunol* 1991;**88**:376–84.
- 70 Springall DR, Howarth PH, Counthan H, Djukanovic R, Holgate ST, Polak JM. Endothelin immunoreactivity of airway epithelium in asthmatic patients. *Lancet* 1991;**337**:697–701.
- 71 Murch SH, Braegger CP, Sessa WC, MacDonald TT. High endothelin-1 immunoreactivity in Crohn's disease and ulcerative colitis. *Lancet* 1992;**339**:381–5.
- 72 Rachmilewitz D, Eliakim R, Ackerman Y, Karmeli F. Colonic endothelin-1 immunoreactivity in active ulcerative colitis. Letter to the Editor. *Lancet* 1992;**339**:1062.
- 73 Wagner OF, Christ G, Wojita J, *et al.* Polar secretion of endothelin-1 by cultured endothelial cells. *J Biol Chem* 1992;**267**: 16066–88.
- 74 Nishikibe M, Ikada M, Tsuchida S, *et al.* Antihypertensive effect of a newly synthesized endothelin antagonist, BQ-123, in genetic hypertension models. (Abstract) *J Hypertens* 1992;**10**(suppl 4): P53.
- 75 McMahon EG, Palomo MA, Moore WM. Phosphoramidon blocks the pressor activity of big endothelin-1 (1-39) and lowers blood pressure in spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1991;**17**(suppl 7):26–8.
- 76 Bunchman TE, Brookshire CA. Cyclosporine-induced synthesis of endothelin by cultured human endothelial cells. *J Clin Invest* 1991;**88**:310–4.
- 77 Perico N, Dadan J, Remuzii G. Endothelin mediates the renal vasoconstriction induced by cyclosporine A-induced nephrotoxicity. *Eur J Pharmacol* 1990;**187**:113–6.

- 78 Kon V, Badr KF. Biological actions and pathophysiological significance of endothelin in the kidney. *Kidney Int* 1991;**40**: 1-12.
- 79 Fogo A, Hellings SE, Inagami T, Kon V. Endothelin receptor antagonism is protective in in vivo acute cyclosporine toxicity. *Kidney Int* 1992;**42**:770-4.
- 80 Firth JD, Ratcliffe PJ, Raine AEG, Ledingham JGG. Endothelin: an important factor in acute renal failure? *Lancet* 1988;ii: 1179-81.
- 81 Tomita K, Ujie K, Nakanishi T, *et al.* Plasma endothelin levels in patients with acute renal failure. *N Engl J Med* 1990;**321**: 1127.
- 82 Marumo F, Tomita K, Sasaki S, Akiba T, Hirata Y. Endothelin and renal failure. *Int J Artif Org* 1991;**14**:259-61.
- 83 Shigeno T, Mima T, Yanagisawa M, *et al.* Possible role of endothelin in the pathogenesis of cerebral vasospasm. *J Cardio-vasc Pharmacol* 1991;**17**(suppl 7):S480-3.
- 84 Matsumura Y, Ikegawa R, Suzuki Y, *et al.* Phosphoramidon prevents cerebral vasospasm following subarachnoid hemorrhage in dogs: The relationship to endothelin-1 levels in the cerebrospinal fluid. *Life Sci* 1991;**49**:841-8.
- 85 Clozel M, Watanabe H. BQ-123, a peptidic endothelin ETA receptor antagonist, prevents the early cerebral vasospasm following subarachnoid hemorrhage after intracisternal but not intravenous injection. *Life Sci* 1993;**52**:825-34.
- 86 Watanabe T, Suzuki N, Shimamoto N, Fujino M, Imada A. Contribution of endogenous endothelin to the extension of myocardial infarct size in rats. *Circ Res* 1991;**69**:370-7.
- 87 Moore K, Wendon J, Frazer M, Karani J, Williams R, Badr K. Plasma endothelin immunoreactivity in liver disease and the hepatorenal syndrome. *N Engl J Med* 1992;**327**:1774-8.

