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Role of Endothelin-1 in Clinical Hypertension 20 Years On

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Hypertension is the most common risk factor worldwide for cardiovascular morbidity and mortality.^{1,2} Currently it is estimated that a quarter of the world's adult population is hypertensive, and this number is projected to increase to $\approx 30\%$ by 2025.¹ Although, there exist a number of drug therapies for hypertension, blood pressure (BP) control to target is still only achieved in $\approx 30\%$ of patients.³ Over the last 20 years, novel licensed therapies have primarily focused on the renin-angiotensin-aldosterone system. Endothelin (ET) receptor antagonism represents an innovative, but as yet only partially explored, alternative approach in the management of hypertension.

A review in *Hypertension* 10 years ago outlined the potential role that ET-1 may play in the development of hypertension,⁴ as proposed by Yanagisawa et al in their original *Nature* article in 1988.⁵ This largely focused on preclinical data because, at that time, there was only 1 published study of ET receptor antagonism in patients with essential hypertension.⁶ There were also few data that focused on the relative benefits of selective or mixed ET blockade. Finally, the lack of longer-term data on safety and tolerability for these drugs made their place in the antihypertensive armamentarium unclear. In this review we aim to answer many of these questions and outline some of the key findings in this field from the last decade.

Biology of the ET System

The ET family consists of three 21-amino acid peptides (ET-1, ET-2, and ET-3) with powerful vasoconstrictor and pressor properties.⁷ Of the 3 peptides, ET-1 is the major vascular isoform and of most importance in the cardiovascular system.⁸ The gene product is the 212-amino acid prepro-ET-1. This is cleaved to big ET-1, after which an ET-converting enzyme (ECE) catalyzes the generation of the biologically active ET-1 and a C-terminal fragment.

ET-1 acts by binding to 2 distinct receptors, the ET_A and the ET_B receptors (ET_AR and ET_BR).^{9,10} ET receptors are expressed by a wide variety of cells and tissues. Within the vasculature, ET_AR and ET_BR, located on vascular smooth muscle cells, mediate the vasoconstrictor effects of ET-1.¹¹

ET_BRs are also found on vascular endothelial cells, where their activation results in vasodilation mediated mainly by NO.^{12,13}

In addition, ET_BRs have a major role in the clearance of circulating ET-1. The plasma half-life of ET-1 in health is ≈ 1 minute,¹⁴ with removal through receptor- and nonreceptor-mediated mechanisms. ET-1 binds to ET_BR, with subsequent ligand-receptor complex internalization and intracellular degradation accounting for the majority of clearance, particularly in the pulmonary circulation,¹⁵ although the splanchnic and renal circulations also contribute.¹⁶ Therefore, a reduction in ET_BR number, or ET_BR blockade, may reduce ET-1 clearance, increasing plasma concentrations without altering production. For this reason and, importantly, because most ET-1 is released albuminally, plasma concentrations of ET-1 do not accurately reflect ET-1 production.

Early in vitro^{17–20} and in vivo²¹ experiments supported a role for ET_BRs in the renal handling of salt and water and, thus, in the regulation of BP. The last decade has seen a number of elegant animal studies focusing on ET_B-mediated natriuresis and diuresis.^{22–24} These experiments suggest that collecting duct-derived ET-1 may mediate natriuresis and diuresis through actions on epithelial rather than endothelial ET_BRs. Interestingly, these data provide insight into the role of renal ET_BRs in salt and water regulation, while not excluding a contribution from extrarenal ET_BRs.²⁵

There are, as yet, no studies of the role of the ET_BR in salt and water balance in humans. However, salt-sensitive hypertension is common in black subjects, and this population has been shown to have higher plasma ET-1 concentrations than white hypertensive subjects and to have enhanced ET_A-dependent vasoconstrictor tone.²⁶ Additional clinical studies in this area would not only provide important information for conditions such as salt-sensitive hypertension but may also provide an explanation for the fluid retention that is often seen as an adverse effect of treatment with ET receptor antagonists.

ET Receptor Antagonists

The last 10 years have seen the clinical development of a number of selective ET_AR and mixed ET_{A/B}R antagonists (see

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Table in Reference 28). The difference between selective and mixed antagonists, however, is not pharmacologically well defined, making antagonist studies difficult to interpret. Selectivity is usually calculated from *in vitro* competitive receptor assays, but results may vary dependent on conditions. “Mixed” antagonists in clinical trials are still selective for the ET_AR, but the ratio of ET_A:ET_B affinity is generally <100-fold selective for ET_A over ET_B compared with ≥100-fold for ET_A selective agents.²⁷ Thus, the degree of receptor selectivity achieved by any particular drug may depend on the dose used, with higher doses of modestly selective antagonists potentially no longer providing selective pharmacological blockade. Unfortunately, there have been no studies in humans to determine the functional selectivity of any of the so-called selective antagonists.

Lack of ET_AR selectivity may account for the disappointing results observed in the clinical trials with ET antagonists in chronic heart failure. Although the case for activation of the ET system in chronic heart failure is clear, and the animal models and early clinical studies were extremely promising, the longer-term data did not live up to expectations. To date, 4 large multicenter, double-blind trials have been completed. The mixed antagonist bosentan was evaluated in both the Research on Endothelin Antagonism in Chronic Heart Failure (REACH-1) and Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure (ENABLE) studies. The former was terminated early because of adverse effects (elevated liver enzymes) attributed to the high dose of drug used (≤500 mg bd of bosentan). Consequently, ENABLE used a lower dose (125 mg bd) with a longer follow-up period. No benefits of bosentan treatment were observed, with an excess of adverse events in the treatment arm. These disappointing results were mirrored in the Enrasentan and Cooperative Randomised Evaluation (ENCOR) study with the mixed antagonist enrasentan (≈100-fold ET_A selective)²⁸ and the Endothelin A Receptor Antagonist Trial in Heart Failure (EARTH) Study, which used the relatively ET_A-selective antagonist darusentan (≈150-fold ET_A selective).²⁹ All of these studies showed a rise in circulating ET-1,^{29,30} whereas plasma ET-1 might be expected to have fallen with hemodynamic improvement. All have used either mixed antagonists or may have used sufficiently high doses of selective ET_A antagonist to block the ET_BR (as suggested by the rise in plasma ET-1). Indeed, it may be that a truly ET_A selective approach has not yet been studied in patients with chronic heart failure. Interestingly, a highly selective ET_A agent, sitaxsentan, caused a decrease in plasma ET-1 concentrations in chronic heart failure patients,³¹ and there are now studies with this compound focusing on diastolic heart failure,³² and their results are eagerly awaited.

Pulmonary arterial hypertension remains the only licensed indication for ET receptor antagonists. Currently, the 3 antagonists clinically available (in the United States, Europe, or both) are the selective ET_A antagonists sitaxsentan and ambrisentan and the mixed antagonist bosentan. Although the experimental data are conflicting,^{33–35} the clinical studies suggest that both selective ET_A and mixed ET_{A/B} approaches are beneficial,^{36,37} although studies have not been designed to show a survival benefit. Furthermore, it is difficult to ascer-

tain whether these agents provide equivalent ET_AR antagonism, so drawing conclusions about whether ET_BR blockade provides additional benefit is difficult. There are also, at present, no robust head-to-head clinical trials comparing selective ET_A and mixed ET_{A/B} receptor antagonism.

ECE inhibitors provide another potentially exciting method of blocking ET-1 activity, especially if combined with angiotensin-converting enzyme (ACE) and neutral endopeptidase inhibition. These agents would act as mixed ET_{A/B} receptor antagonists without affecting ET_BR-mediated ET-1 clearance. However, there may be problems with combining ECE, ACE, and neutral endopeptidase inhibition. First, there exist a number of non-ECE ET-1-generating enzymes,³⁸ and, second, there is a potential risk of angioedema with these drugs. There has been difficulty synthesizing compounds with a sufficiently high degree of inhibition at each of these enzymes, and, apart from data for SLV-306,³⁹ these agents have been slow to emerge.

Essential Hypertension

Initial evidence of a pressor action of ET-1 led to the speculation that it might be implicated in hypertension.⁵ Production of vascular ET-1 is increased in some but not all of the animal models of hypertension.⁴ Those models where ET-1 production is increased (mostly, but not exclusively, salt-dependent types) are associated with increased vascular growth and a response to both selective and mixed ET receptor antagonism composed of not only a modest reduction in BP but also a marked regression of vascular growth.⁴ This was initially demonstrated in deoxycorticosterone acetate-salt hypertensive rats^{40,41} and has since been unambiguously confirmed in a mouse model with endothelium-restricted overexpression of human prepro-ET-1, which exhibits inward hypertrophic remodeling of the resistance arteries, and vascular inflammation, in the absence of an elevation in BP.^{42,43}

An early study in humans compared plasma ET-1 concentrations among subjects with pheochromocytoma (in the presence or absence of hypertension), those with essential hypertension, and healthy control subjects. Significantly higher levels of ET-1 were seen in those with pheochromocytoma compared with the 2 other groups, and, among these, the presence of hypertension was associated with the highest plasma ET-1 concentrations. These findings supported a role for ET-1 in the development of clinical hypertension,⁴⁴ although many solid cancers generate ET-1. In agreement with preclinical data, which suggested that the ET system was primarily activated in the more severe rodent models of BP elevation (such as deoxycorticosterone acetate-salt, Dahl salt-sensitive, and stroke-prone spontaneously hypertensive rats), the increased prepro-ET-1 message is found in the endothelium of small arteries of patients with moderate-to-severe hypertension.⁴⁵ ET-1 message and protein are also increased in the vascular smooth muscle cells from larger elastic and muscular arteries of hypertensive patients.⁴⁶ In local studies, Cardillo et al⁴⁷ have suggested increased vascular ET system activity in patients with hypertension compared with normotensive control subjects and a greater forearm vascular response to mixed receptor antagonism compared with selective ET_A antagonism, consistent with an

increased importance of vascular smooth muscle vasoconstrictor ET_BR in hypertension. Others have differed in their results.⁴⁸ In both mice and rats, pharmacological or genetic inhibition of ET_BR activity results in a severe form of hypertension that depends on the extent of salt intake.^{49,50} Although it is easy to speculate that some forms of clinical hypertension may be attributable to a lack of ET_BR function, this idea has yet to be explored in clinical studies.

Few studies have investigated the longer-term antihypertensive effects of ET receptor antagonism in humans. Bosentan treatment for 4 weeks reduced BP in essential hypertensive patients as much as 20 mg of enalapril (\approx 6 mm Hg).⁶ Importantly, this reduction was achieved without activation of the sympathetic nervous system or the renin-angiotensin-aldosterone system. In another study, 6 weeks of darusentan, a selective ET_AR antagonist, was also effective in lowering both systolic and diastolic BPs compared with placebo.⁵¹ There are currently no clinical studies that directly compare selective and mixed ET receptor antagonism at systemic doses in the treatment of hypertension, although both approaches clearly reduce BP.

Hypertension Associated With Chronic Kidney Disease

Renal function may influence the relationship between ET-1 and hypertension. First, as renal function declines, plasma ET-1 levels increase.^{52,53} It remains unclear whether the rise in plasma ET-1 in chronic kidney disease (CKD) is because of biologically active or simply immunologically competent peptide, but infusion of exogenous ET-1 to bilaterally nephrectomized rats results in a increased plasma half-life of ET-1 and a prolonged rise in BP compared with sham-operated rats⁵⁴ consistent with the idea that elevated plasma ET-1 concentrations in CKD may contribute to hypertension. Second, there is an upregulation of renal ET-1 production in CKD,⁵⁵ as reflected by increased urinary ET-1 excretion.^{53,56} The effects of exogenous ET-1 on the renal vasculature are vasoconstriction, activating the renin-angiotensin-aldosterone system, and causing salt and water retention, both of which have the potential to raise BP. Animal data suggest that, in CKD, the renal vasculature may be more sensitive to the vasoconstrictor effects of ET-1 than in normal kidneys.⁵⁷ Thus, an amplification of the renal vasoconstrictor effects of ET-1, promoting hypertension, could be envisaged in CKD.

Preclinical data suggest that, in CKD, selective ET_AR antagonism may be preferential to mixed blockade.^{58,59} This is supported by clinical studies. In hypertensive patients with CKD, the systemic vasodilation seen with acute ET_AR blockade (associated with a reduction in BP of \approx 10 mm Hg) is attenuated by concomitant ET_BR antagonism, suggesting that, at least in this disease state, vasoconstrictor ET_BR activity is less important than ET_B vasodilatory function.⁶⁰ It is noteworthy, however, that these were acute studies, and the effects of chronic dosing in patients with CKD remain unknown. Interestingly, most of the patients studied were already taking ACE inhibitors, and data from healthy subjects suggest a synergy between ET_AR antagonism and ACE inhibition that is not only dependent on an unblocked ET_BR but is also associated with a significant natriuresis.⁶¹ This is

important clinically because patients with CKD are generally prescribed ACE inhibitors, not only for BP control but also for their renoprotective effects. Emerging data in diabetic nephropathy suggest a role for ET antagonism, on top of standard therapy, in reducing proteinuria⁶² and thereby potentially offering longer-term renal protection. There are currently no studies reported in nondiabetic, proteinuric CKD, although acute dosing studies have shown a reduction in effective filtration fraction,⁶⁰ which would be expected to translate to a reduction in intraglomerular pressure and, consequently, proteinuria.

Hypertension and the Metabolic Syndrome

Insulin resistance and compensatory hyperinsulinemia contribute to the hypertension characteristic of the metabolic syndrome,⁶³ in which data from *in vitro*, animal, and human studies suggest that ET-1 plays a role.⁶⁴ In health, insulin promotes production of both ET-1 and NO from the vascular wall. In subjects with insulin resistance, NO release is impaired,^{65,66} whereas ET-1 production is preserved.^{67,68} Indeed, circulating ET-1 concentrations are elevated in patients with insulin resistance,⁶⁹ and this is not thought to reflect reduced ET-1 clearance.⁷⁰ Thus, in states of insulin resistance, an imbalance between the ET-1/NO systems favors vasoconstriction, which may be further amplified by a reduction in the inhibitory effects of NO on ET-1 production.⁷¹

Animal studies support a role for ET-1 in the vasoconstrictive response to insulin and thereby development of hypertension in insulin-resistant states.^{70,72,73} Juan et al⁷⁴ studied 2 groups of rats, 1 receiving a continuous insulin infusion, the other saline. The former was characterized by hyperinsulinemia and a gradual development of insulin resistance and hypertension. They also had higher plasma ET-1 concentrations than control animals. Both groups were then treated with daily intraperitoneal saline or a selective ET_AR antagonist. The 2 groups receiving the intraperitoneal ET_A antagonist had similarly reduced BP levels, whereas, of those receiving intraperitoneal saline, hyperinsulinemic rats had a significantly higher BP than controls.

Human studies also suggest that ET-1 contributes to hypertension in insulin-resistant states. Cardillo et al⁷⁵ showed an increase in forearm blood flow with both selective ET_A and mixed ET_{A/B}R antagonism in patients with type 2 diabetes compared with healthy individuals. Similarly, mixed ET_{A/B}R antagonism produced both a significant increase in forearm blood flow, as well as a potentiation of endothelium-dependent vasodilation in hypertensive patients compared with control subjects.⁷⁶ Taken together, these data suggest an increase in ET-1 activity in type 2 diabetes and hypertension compared with health. Although no measurements of insulin resistance were taken in these studies, one could hypothesize this as a potential mechanism for the upregulation in ET-1 activity. This is supported by a recent study that included subjects with varying degrees of insulin resistance and showed mixed ET_{A/B}R antagonism to significantly increase forearm endothelium-dependent vasodilation in insulin-resistant but not insulin-sensitive subjects.⁷⁷ At present, there are no systemic studies using either selective ET_A or mixed ET_{A/B}R antagonists

that examine hemodynamics in hypertensive patients with insulin resistance.

Hypertension and Broader Cardiovascular Risk

Endothelial Dysfunction

The endothelium is a crucial regulator of vascular tone,⁷⁸ and its function is impaired both in patients with hypertension and patients at risk of hypertension, with a shift toward reduced vasodilation, associated with a proinflammatory and prothrombotic state. Endothelial dysfunction is recognized to be a key early determinant in the progression to atherosclerosis and is now well established to be independently associated with increased cardiovascular risk.⁷⁹ Mice with endothelium-restricted overexpression of human prepro-ET-1 exhibit increased oxidative stress and endothelial dysfunction as demonstrated by an impaired vasodilator response to acetylcholine.⁴² Animal models of endothelial dysfunction have shown that antagonism of the ET system, predominantly with selective ET_AR antagonists, improves NO-mediated endothelial function,^{80–82} suggesting that ET-1, acting via the ET_AR, is involved in the pathogenesis of endothelial dysfunction.

Atherosclerosis

Hypertension contributes to the development of atherosclerosis. In addition to its effects on BP, ET-1 is proinflammatory⁸³ and is implicated in the development of atherosclerosis. Both ET_A and ET_BR are highly expressed in smooth muscle cells and foamy macrophages in atherosclerotic models.⁸⁴ Increased expression of ET-1 and ECE is seen in human arteries at different stages of atherosclerosis,^{46,85} and high levels of ET-1 have been found in human atherosclerotic lesions.^{46,85–87} Furthermore, plasma ET-1 concentrations correlate positively with the degree of atherosclerosis present.⁸⁶ Importantly, not only is restoration of the impaired activity of the NO system seen after ET receptor antagonism in a range of animal models of atherosclerosis,^{80,81,84} but so too is a reversal of atherosclerotic lesion development. Thus, ET antagonists reduce the activity of the ET system, increase NO bioavailability, and slow the progression of atherosclerosis.

Several animal models have shown benefit of both selective ET_A and mixed ET_{A/B}R antagonism in the development of atherosclerotic lesions.^{80,84,88,89} In humans, there are data that support a role for the ET_AR in coronary vascular tone and endothelial dysfunction in coronary artery disease^{90,91}; however, these were acute dosing studies, and there are as yet no randomized or chronic dosing clinical trials in patients with atherosclerosis.

Arterial Stiffness and Isolated Systolic Hypertension

Arterial stiffness is linked to endothelial dysfunction,⁹² and the 2 commonly coexist in patients at increased cardiovascular risk. A number of interventions that reduce arterial stiffness also improve endothelial function.⁹² To date, there have been few studies addressing the relationship between these 2 markers of cardiovascular disease after treatment. However, both animal and human studies suggest that the endothelium is an important regulator of arterial stiffness.

Basal endogenous NO generation decreases arterial stiffness in animals⁹³ and humans.^{94–96} By contrast, ET-1 increases arterial stiffness, as shown in the ET-1–overexpressing mouse,⁴² in which vascular stiffness is increased in association with increased collagen deposition, and these effects are independent of BP. Furthermore, exogenous ET-1 has been shown recently to increase arterial stiffness in humans,⁹⁷ and studies using ET_AR antagonism have shown that endogenous ET-1 is responsible for maintaining arterial stiffness⁹⁸ to a greater extent than NO.⁹³ Thus, in endothelial dysfunction, where NO is downregulated and ET-1 upregulated, the balance will likely shift in favor of increased arterial stiffness.

Isolated systolic hypertension is common in the elderly and is associated with increased arterial stiffness. Treatments that not only lower BP but also reduce vascular stiffness would be particularly attractive in isolated systolic hypertension, considering that, in patients with CKD and hypertension, a greater survival is seen when both BP and arterial stiffness are reduced, as opposed to just BP alone.⁹⁹ Currently there are no studies of the longer-term effects of ET receptor antagonism on arterial stiffness in any patient group.

Proteinuria

Proteinuria is a feature of hypertensive renal damage. Although traditionally a risk factor for renal disease progression,¹⁰⁰ proteinuria is now an established independent risk factor for global cardiovascular risk. Albuminuria is incrementally associated with increased cardiovascular risk in both individuals with pre-existing risk (such as hypertensive patients)¹⁰¹ and in individuals with no known risk factors.¹⁰² This is true even in the presence of normal renal function.¹⁰³ Importantly, in patients with hypertension, reduction of albuminuria confers cardiovascular protection.¹⁰¹

Through its hemodynamic effects, ET-1 contributes to the development of proteinuria.⁸³ Both acute⁶⁰ and chronic¹⁰⁴ selective ET_A blockade have been shown to reduce proteinuria in patients with diabetic¹⁰⁴ and nondiabetic⁶⁰ proteinuric CKD, and these effects are abolished by concomitant ET_BR antagonism.⁶⁰ As yet, it remains unclear to what extent these effects are explained by BP reduction alone. However, should ET receptor antagonists be found to have a potential for renal protection in addition to their BP-lowering effects, and this, furthermore, on top of standard therapies,^{61,104} they would clearly be an attractive therapeutic option for patients with CKD.

Tolerability and Safety of ET Receptor Antagonists

Adverse effects of ET receptor antagonists in clinical trials are common. The most frequently reported clinical adverse events are headache, peripheral edema, dizziness, nausea, nasal congestion, and dyspnea. These appear to be a class effect, likely relate to vasodilation, and do not often lead to treatment withdrawal. The mechanism of peripheral edema with ET receptor antagonism remains unclear. ET-1 acts in the renal tubule via the ET_BR to promote natriuresis and diuresis. Thus, peripheral edema associated with vasodilation could be aggravated by mixed ET_{A/B} antagonists because of the reported ET_B-mediated downregulation of the renal tubular epithelial sodium channel.¹⁰⁵ However, this issue remains

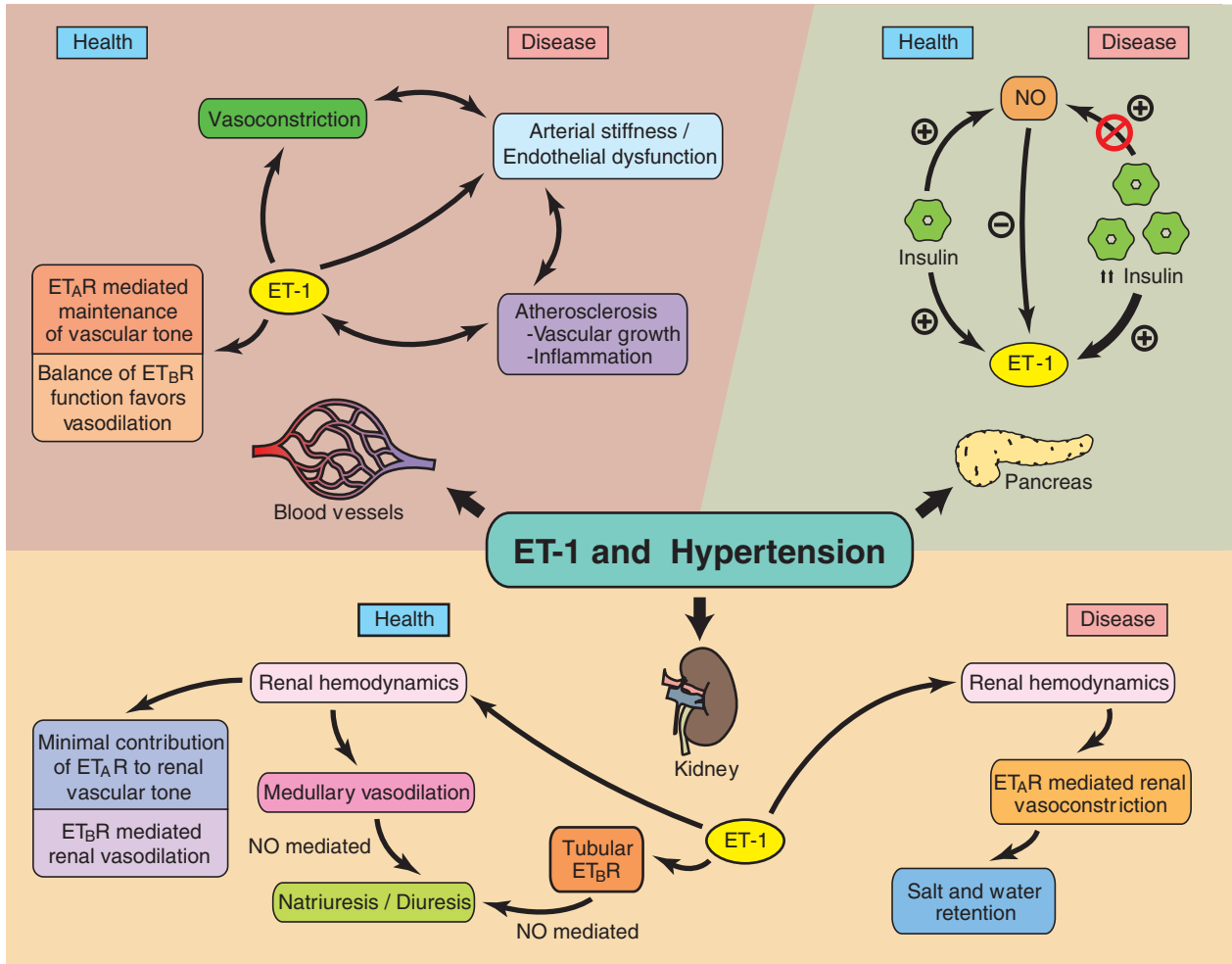


Figure. The figure shows the role of ET-1 in hypertension largely based on data from human studies. Within healthy blood vessels, ET-1 maintains vascular tone via the ET_AR, with the balance of ET_BR activity favoring vasodilation. In disease, ET-1 promotes hypertension and cardiovascular disease through a number of mechanisms. In the healthy kidney, the ET_BR plays a role in tonic vasodilation with little contribution of the ET_AR to renal vascular tone. Both increases in medullary blood flow and direct actions of ET-1 on the ET_BR may mediate natriuresis and diuresis. In chronic kidney disease, ET_AR-mediated renal vasoconstriction leads to salt and water retention, promoting the development of hypertension. Finally, in health, insulin promotes release of both ET-1 and NO. In states of insulin resistance, NO release is impaired, whereas ET-1 production is increased.

unresolved, because in clinical trials fluid retention appears to occur with both selective ET_A⁶² and mixed ET_{A/B}R antagonists. This may be because tubular ET_ARs are also involved in the development of fluid retention, but this issue remains unresolved. Another possible explanation for this edema may relate to the characteristics of the patients treated. ET-1 has an inotropic effect.¹⁶ Those with cardiac dysfunction may depend on ET-1-mediated inotropy, and so blockade of this could lead to cardiac decompensation and fluid accumulation. Until the issue of fluid retention is resolved, the ideal target population for ET receptor antagonists will remain an issue.

Liver toxicity is also a dose-dependent and, possibly, class effect. This has led not only to intense liver enzyme surveillance but also to an active search for the lowest efficacious drug dose in clinical trials. It remains to be firmly established, however, whether liver toxicity varies among ET receptor antagonists at equipotent doses. Finally, all of the ET antagonists are contraindicated in pregnancy, because they are

teratogenic. Because these drugs have only been available for a relatively short time, for the orphan indication of pulmonary artery hypertension their balance of risks and benefits remains incompletely understood, and longer-term observational studies are needed.

Perspectives

Current data support a role for ET receptor antagonism in the management of essential hypertension and the hypertension associated with systemic disorders (see Figure). In addition to those described here, data are emerging in a number of other areas where BP control is often a problem, eg, after transplantation. ET-1 has been implicated in the hypertension-complicating heart,¹⁰⁶ lung,¹⁰⁷ and kidney¹⁰⁸ transplants, and although animal data suggest a role for ET receptor antagonists in treating this hypertension,¹⁰⁹ there are currently few human data. Although unlikely to be considered as first-line treatment, ET receptor antagonists are a promising new and innovative drug class to add to the antihypertensive arma-

mentarium. Currently, the selective ET_AR antagonist darusentan is being evaluated for the treatment of resistant hypertension, in agreement with data that the system is activated in severe forms of hypertension. Importantly, ET_AR-selective antagonists may have a particular role in the treatment of high-risk patients, such as those with salt-sensitive hypertension, those with progressive CKD, those who develop hypertension after transplantation, or those with hypertension as part of the metabolic syndrome or diabetes mellitus.

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References

- Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet*. 2005;365:217–223.
- Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. *Lancet*. 2008;371:1513–1518.
- Hajjar I, Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the United States, 1988–2000. *JAMA*. 2003;290:199–206.
- Schiffrin EL. State-of-the-Art lecture. Role of endothelin-1 in hypertension. *Hypertension*. 1999;34:876–881.
- Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*. 1988;332:411–415.
- Krum H, Viskoper RJ, Lacourciere Y, Budde M, Charlon V. The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. *N Engl J Med*. 1998;338:784–790.
- Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyachi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci U S A*. 1989;86:2863–2867.
- Haynes WG, Webb DJ. The endothelin family of peptides: local hormones with diverse roles in health and disease? *Clin Sci (Lond)*. 1993;84:485–500.
- Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature*. 1990;348:730–732.
- Sakurai T, Yanagisawa M, Takuya Y, Miyazaki H, Kimura S, Goto K, Masaki T. Cloning of a cDNA encoding a non-isopeptide selective subtype of the endothelin receptor. *Nature*. 1990;348:732–735.
- Davenport AP, O'Reilly G, Molenaar P, Maguire JJ, Kuc RE, Sharkey A, Bacon CR, Ferro A. Human endothelin receptors characterized using reverse transcriptase-polymerase chain reaction, in situ hybridization, and subtype-selective ligands BQ123 and BQ3020: evidence for expression of ETB receptors in human vascular smooth muscle. *J Cardiovasc Pharmacol*. 1993;22:S22–S25.
- Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, Webb DJ. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation*. 1998;97:752–756.
- Newby DE, Strachan FE, Webb DJ. Abnormal endothelin B receptor vasomotor responses in patients with Hirschprung's disease. *Q J Med*. 2002;95:159–163.
- Gasic S, Wagner OF, Vierhapper H, Nowotny P, Waldhausl W. Regional haemodynamic effects and clearance of endothelin-1 in humans: renal and peripheral tissues may contribute to overall disposal of the peptide. *J Cardiovasc Pharmacol*. 1992;19:176–180.
- Dupuis J, Stewart DJ, Cernacek P, Gosselin G. Human pulmonary circulation is an important site for both clearance and production of endothelin-1. *Circulation*. 1996;94:1278–1284.
- Attina T, Camidge R, Newby DE, Webb DJ. Endothelin antagonism in pulmonary hypertension, heart failure, and beyond. *Heart*. 2005;91:825–831.
- Tomita K, Nonoguchi H, Terada Y, Marumo F. Effects of ET-1 on water and chloride transport in cortical collecting ducts of the rat. *Am J Physiol*. 1993;264:690–696.
- Kohan DE, Padilla E. Osmolar regulation of endothelin-1 production by rat inner medullary collecting duct. *J Clin Invest*. 1993;91:1235–1240.
- Gallego MS, Ling BN. Regulation of amiloride-sensitive Na⁺ channels by endothelin-1 in distal nephron cells. *Am J Physiol*. 1996;271:451–460.
- Plato CF, Pollock DM, Garvin JL. Endothelin inhibits thick ascending limb chloride flux via ET(B) receptor-mediated NO release. *Am J Physiol*. 2000;279:326–333.
- Harris PJ, Zhuo J, Mendelsohn FA, Skinner SL. Haemodynamic and renal tubular effects of low doses of endothelin in anaesthetized rats. *J Physiol*. 1991;433:25–39.
- Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE. Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. *J Clin Invest*. 2004;114:504–511.
- Bagnall AJ, Kelland NF, Gulliver-Sloan F, Davenport AP, Gray GA, Yanagisawa M, Webb DJ, Kotelevtsev YV. Deletion of endothelial cell endothelin B receptors does not affect blood pressure or sensitivity to salt. *Hypertension*. 2006;48:286–293.
- Ge Y, Bagnall A, Stricklett PK, Strait K, Webb DJ, Kotelevtsev Y, Kohan DE. Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention. *Am J Physiol*. 2006;291:1274–1280.
- Ohkita M, Wang Y, Nguyen ND, Tsai YH, Williams SC, Wiseman RC, Killen PD, Li S, Yanagisawa M, Garipey CE. Extrarenal ETB plays a significant role in controlling cardiovascular responses to high dietary sodium in rats. *Hypertension*. 2005;45:940–946.
- Campia U, Cardillo C, Panza JA. Ethnic differences in the vasoconstrictor activity of endogenous endothelin-1 in hypertensive patients. *Circulation*. 2004;109:3191–3195.
- IUPHAR Database. Endothelin receptors. Available at: <http://www.iuphar-db.org/GPCR/ReceptorDisplayForward?receptorID=2263>. Accessed July 23, 2008.
- Battistini B, Berthiaume N, Kelland NF, Webb DJ, Kohan DE. Profile of past and current clinical trials involving endothelin receptor antagonists: the novel “-sentan” class of drug. *Soc Exp Biol Med*. 2006;231:653–695.
- Anand I, McMurray J, Cohn JN, Konstam MA, Notter T, Quitzau K, Ruschitzka F, Luscher TF, EARTH investigators. Long-term effects of darusentan on left-ventricular remodelling and clinical outcomes in the EndothelinA Receptor Antagonist Trial in Heart Failure (EARTH): randomised, double-blind, placebo-controlled trial. *Lancet*. 2004;364:327–354.
- Luscher TF, Enseleit F, Pacher R, Mitrovic V, Schulze MR, Willenbrock R, Dietz R, Rousson V, Hurlimann D, Philipp S, Notter T, Noll G, Ruschitzka F. Hemodynamic and neurohumoral effects of selective endothelin A (ET(A)) receptor blockade in chronic heart failure: the Heart Failure ET(A) Receptor Blockade Trial (HEAT). *Circulation*. 2002;106:2666–2672.
- Givertz MM, Colucci WS, LeJemtel TH, Gottlieb SS, Hare JM, Slawsky MT, Leier CV, Loh E, Nicklas JM, Lewis BE. Acute endothelin A receptor blockade causes selective pulmonary vasodilation in patients with chronic heart failure. *Circulation*. 2000;101:2922–2927.
- ClinicalTrials.gov. A study of sitaxsentan sodium in subjects with diastolic heart failure. Available at: <http://www.clinicaltrials.gov/ct2/show/NCT00303498?term=sitaxsentan&rank=4>. Accessed July 23, 2008.
- Eddahibi S, Raffestin B, Clozel M, Levame M, Adnot S. Protection from pulmonary hypertension with an orally active endothelin receptor antagonist in hypoxic rats. *Am J Physiol*. 1995;268:828–835.

34. Ivy DD, Parker TA, Abman SH. Prolonged endothelin B receptor blockade causes pulmonary hypertension in the ovine fetus. *Am J Physiol*. 2000;279:758–765.
35. Ivy D, McMurtry IF, Yanagisawa M, Garipey CE, Le Cras TD, Gebb SA, Morris KG, Wiseman RC, Abman SH. Endothelin B receptor deficiency potentiates ET-1 and hypoxic pulmonary vasoconstriction. *Am J Physiol*. 2001;280:1040–1048.
36. Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, Pulido T, Frost A, Roux S, Leconte I, Landzberg M, Simonneau G. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med*. 2002;346:896–903.
37. Barst RJ, Langleben D, Frost A, Horn EM, Oudiz R, Shapiro S, McLaughlin V, Hill N, Tapson VF, Robbins IM, Zwicke D, Duncan B, Dixon RA, Frumkin LR. Sitaxsentan therapy for pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2004;169:441–447.
38. Davenport AP, Maguire JJ. Endothelin. *Handb Exp Pharmacol*. 2006;295–329.
39. Tabrizchi R. SLV-306. Solvay. *Curr Opin Investig Drugs*. 2003;4:329–332.
40. Lariviere R, Thibault G, Schiffrin EL. Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. *Hypertension*. 1993;21:294–300.
41. Li JS, Lariviere R, Schiffrin EL. Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats. Evidence for a role of endothelin in vascular hypertrophy. *Hypertension*. 1994;24:183–188.
42. Amiri F, Virdis A, Neves MF, Iglarz M, Seidah NG, Touyz RM, Reudelhuber TL, Schiffrin EL. Endothelin-restricted overexpression of human endothelin-1 causes vascular remodeling and endothelial dysfunction. *Circulation*. 2004;110:2233–2240.
43. Amiri F, Paradis P, Reudelhuber TL, Schiffrin EL. Vascular inflammation in absence of blood pressure elevation in transgenic murine model overexpressing endothelin-1 in endothelial cells. *J Hypertens*. 2008;26:1102–1109.
44. Oishi S, Sasaki M, Sato T. Elevated immunoreactive endothelin levels in patients with pheochromocytoma. *Am J Hypertens*. 1994;7:717–722.
45. Schiffrin EL, Deng LY, Svntek P, Day R. Enhanced expression of endothelin-1 gene in resistance arteries in severe human essential hypertension. *J Hypertens*. 1997;15:57–63.
46. Rossi GP, Colonna S, Pavan E, Albertin G, Della Rocca F, Gerosa G, Casarotto D, Sartore S, Pauletto P, Pessina AC. Endothelin-1 and its mRNA in the wall layers of human arteries ex vivo. *Circulation*. 1999;99:1147–1155.
47. Cardillo C, Kilcoyne CM, Waclawiw M, Cannon RO III, Panza JA. Role of endothelin in the increased vascular tone of patients with essential hypertension. *Hypertension*. 1999;33:753–758.
48. Martin P, Ninio D, Krum H. Effect of endothelin blockade on basal and stimulated forearm blood flow in patients with essential hypertension. *Hypertension*. 2002;39:821–824.
49. Garipey CE, Ohuchi T, Williams SC, Richardson JA, Yanagisawa M. Salt-sensitive hypertension in endothelin-B receptor-deficient rats. *J Clin Invest*. 2000;105:925–933.
50. Pollock DM, Pollock JS. Evidence for endothelin involvement in the response to high salt. *Am J Physiol*. 2001;281:144–150.
51. Nakov R, Pfarr E, Eberle S. Darusentan: an effective endothelinA receptor antagonist for treatment of hypertension. *Am J Hypertens*. 2002;15:583–589.
52. Koyama H, Tabata T, Nishizawa Y, Inoue T, Morii H, Yamaji T. Plasma endothelin levels in patients with uraemia. *Lancet*. 1989;1:991–992.
53. Goddard J, Johnston NR, Cumming AD, Webb DJ. Fractional urinary excretion of endothelin-1 is reduced by acute ETB receptor blockade. *Am J Physiol*. 2007;293:1433–1438.
54. Kohno M, Murakawa K, Yasunari K, Yokokawa K, Horio T, Kurihara N, Takeda T. Prolonged blood pressure elevation after endothelin administration in bilaterally nephrectomized rats. *Metab Clin Exp*. 1989;38:712–713.
55. Orisio S, Benigni A, Bruzzi I, Corna D, Perico N, Zoja C, Benatti L, Remuzzi G. Renal endothelin gene expression is increased in remnant kidney and correlates with disease progression. *Kidney Int*. 1993;43:354–358.
56. Zoccali C, Leonardi D, Parlongo S, Mallamaci F, Postorino M. Urinary and plasma endothelin-1 in essential hypertension and in hypertension secondary to renoparenchymal disease. *Nephrol Dial Transplant*. 1995;10:1320–1323.
57. Kanai H, Okuda S, Kiyama S, Tomooka S, Hirakata H, Fujishima M. Effects of endothelin and angiotensin II on renal haemodynamics in experimental mesangial proliferative nephritis. *Nephron*. 1993;64:609–614.
58. Vaneckova I, Kramer HJ, Backer A, Vernerova Z, Opocensky M, Cervenka L. Early endothelin-A receptor blockade decreases blood pressure and ameliorates end-organ damage in homozygous Ren-2 rats. *Hypertension*. 2005;46:969–974.
59. Opocensky M, Kramer HJ, Backer A, Vernerova Z, Eis V, Cervenka L, Certikova Chabova V, Tesar V, Vaneckova I. Late-onset endothelin-A receptor blockade reduces podocyte injury in homozygous Ren-2 rats despite severe hypertension. *Hypertension*. 2006;48:965–971.
60. Goddard J, Johnston NR, Hand MF, Cumming AD, Rabelink TJ, Rankin AJ, Webb DJ. Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure: a comparison of selective and combined endothelin receptor blockade. *Circulation*. 2004;109:1186–1193.
61. Goddard J, Eckhart C, Johnston NR, Cumming AD, Rankin AJ, Webb DJ. Endothelin A receptor antagonism and angiotensin-converting enzyme inhibition are synergistic via an endothelin B receptor-mediated and nitric oxide-dependent mechanism. *J Am Soc Nephrol*. 2004;15:2601–2610.
62. ClinicalTrials.gov. To determine the effects of avosentan on doubling of serum creatinine, end stage renal disease and death in diabetic nephropathy. Available at: <http://www.clinicaltrials.gov/ct/show/NCT00120328>. Accessed July 23, 2008.
63. Sartori C, Scherrer U. Insulin, nitric oxide and the sympathetic nervous system: at the crossroads of metabolic and cardiovascular regulation. *J Hypertens*. 1999;17:1517–1525.
64. Sarafidis PA, Bakris GL. Review: insulin and endothelin: an interplay contributing to hypertension development? *J Clinical Endocrinol Metabol*. 2007;92:379–385.
65. Steinberg HO, Baron AD. Vascular function, insulin resistance and fatty acids. *Diabetologia*. 2002;45:623–634.
66. Sarafidis PA, Lasaridis AN. Actions of peroxisome proliferator-activated receptors-gamma agonists explaining a possible blood pressure-lowering effect. *Am J Hypertens*. 2006;19:646–653.
67. Miller AW, Tulbert C, Puskar M, Busija DW. Enhanced endothelin activity prevents vasodilation to insulin in insulin resistance. *Hypertension*. 2002;40:78–82.
68. Eringa EC, Stehouwer CD, Merlijn T, Westerhof N, Sipkema P. Physiological concentrations of insulin induce endothelin-mediated vasoconstriction during inhibition of NOS or PI3-kinase in skeletal muscle arterioles. *Cardiovasc Res*. 2002;56:464–471.
69. Wolpert HA, Steen SN, Istfan NW, Simonson DC. Insulin modulates circulating endothelin-1 levels in humans. *Metabolism*. 1993;42:1027–1030.
70. Katakam PV, Pollock JS, Pollock DM, Ujhelyi MR, Miller AW. Enhanced endothelin-1 response and receptor expression in small mesenteric arteries of insulin-resistant rats. *Am J Physiol*. 2001;280:522–527.
71. Goligorsky MS, Tsukahara H, Magazine H, Andersen TT, Malik AB, Bahou WF. Termination of endothelin signaling: role of nitric oxide. *J Cell Physiol*. 1994;158:485–494.
72. Verma S, Bhanot S, McNeill JH. Effect of chronic endothelin blockade in hyperinsulinemic hypertensive rats. *Am J Physiol*. 1995;269:2017–2021.
73. Hopfner RL, Hasnadka RV, Wilson TW, McNeill JR, Gopalakrishnan V. Insulin increases endothelin-1-evoked intracellular free calcium responses by increased ET(A) receptor expression in rat aortic smooth muscle cells. *Diabetes*. 1998;47:937–944.
74. Juan CC, Shen YW, Chien Y, Lin YJ, Chang SF, Ho LT. Insulin infusion induces endothelin-1-dependent hypertension in rats. *Am J Physiol*. 2004;287:948–954.
75. Cardillo C, Campia U, Bryant MB, Panza JA. Increased activity of endogenous endothelin in patients with type II diabetes mellitus. *Circulation*. 2002;106:1783–1787.
76. Cardillo C, Campia U, Kilcoyne CM, Bryant MB, Panza JA. Improved endothelin-dependent vasodilation after blockade of endothelin receptors in patients with essential hypertension. *Circulation*. 2002;105:452–456.
77. Shemyakin A, Bohm F, Wagner H, Efendic S, Bavenholm P, Pernow J. Enhanced endothelin-dependent vasodilatation by dual endothelin receptor blockade in individuals with insulin resistance. *J Cardiovasc Pharmacol*. 2006;47:385–390.

78. Endemann DH, Schiffrin EL. Endothelial dysfunction. *J Am Soc Nephrol*. 2004;15:1983–1992.
79. Lerman A, Zeiher AM. Endothelial function: cardiac events. *Circulation*. 2005;111:363–368.
80. Barton M, Haudenschild CC, D'Uscio LV, Shaw S, Munter K, Luscher TF. Endothelin ET(A) receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apolipoprotein E-deficient mice. *Proc Natl Acad Sci U S A*. 1998;95:14367–14372.
81. Best PJM, McKenna CJ, Hasdai D, Holmes DRJ, Lerman A. Chronic endothelin receptor antagonism preserves coronary endothelial function in experimental hypercholesterolaemia. *Circulation*. 1999;99:1747–1752.
82. Bauersachs J, Fraccarollo D, Galuppo P, Widder J, Ertl G. Endothelin-receptor blockade improves endothelial vasomotor dysfunction in heart failure. *Cardiovasc Res*. 2000;47:142–149.
83. Dhaun N, Goddard J, Webb DJ. The endothelin system and its antagonism in chronic kidney disease. *J Am Soc Nephrol*. 2006;17:943–955.
84. Kowala MC, Rose PM, Stein PD, Goller N, Recce R, Beyer S, Valentine M, Barton D, Durham SK. Selective blockade of the endothelin subtype A receptor decreases early atherosclerosis in hamsters fed cholesterol. *Am J Pathol*. 1995;146:819–826.
85. Ihling C, Szombathy T, Bohrmann B, Brockhaus M, Schaefer HE, Loeffler BM. Coexpression of endothelin-converting enzyme-1 and endothelin-1 in different stages of human atherosclerosis. *Circulation*. 2001;104:864–869.
86. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC Jr. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med*. 1991;325:997–1001.
87. Timm M, Kaski JC, Dashwood MR. Endothelin-like immunoreactivity in atherosclerotic human coronary arteries. *J Cardiovasc Pharmacol*. 1995;26:442–444.
88. Babaei S, Picard P, Ravandi A, Monge JC, Lee TC, Cernacek P, Stewart DJ. Blockade of endothelin receptors markedly reduces atherosclerosis in LDL receptor deficient mice: role of endothelin in macrophage foam cell formation. *Cardiovasc Res*. 2000;48:158–167.
89. d'Uscio LV, Barton M, Shaw S, Luscher TF. Chronic ET(A) receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. *Cardiovasc Res*. 2002;53:487–495.
90. Halcox JP, Nour KR, Zalos G, Quyyumi AA. Coronary vasodilation and improvement in endothelial dysfunction with endothelin ET(A) receptor blockade. *Circ Res*. 2001;89:969–976.
91. Halcox JP, Nour KR, Zalos G, Quyyumi AA. Endogenous endothelin in human coronary vascular function. Differential contribution of endothelin receptor types A and B. *Hypertension*. 2007;49:1–8.
92. Oliver JJ, Webb DJ. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol*. 2003;23:554–566.
93. Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP, Cockcroft JR. Nitric oxide regulates local arterial distensibility in vivo. *Circulation*. 2002;105:213–217.
94. Kinlay S, Creager MA, Fukumoto M, Hikita H, Fang JC, Selwyn AP, Ganz P. Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo. *Hypertension*. 2001;38:1049–1053.
95. Wilkinson IB, MacCallum H, Cockcroft JR, Webb DJ. Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. *Br J Clin Pharmacol*. 2002;53:189–192.
96. Schmitt M, Avolio A, Qasem A, McEniery CM, Butlin M, Wilkinson IB, Cockcroft JR. Basal NO locally modulates human iliac artery function in vivo. *Hypertension*. 2005;46:227–231.
97. Vuurmans TJ, Boer P, Koomans HA. Effects of endothelin-1 and endothelin-1 receptor blockade on cardiac output, aortic pressure, and pulse wave velocity in humans. *Hypertension*. 2003;41:1253–1258.
98. McEniery CM, Qasem A, Schmitt M, Avolio AP, Cockcroft JR, Wilkinson IB. Endothelin-1 regulates arterial pulse wave velocity in vivo. *J Am Coll Cardiol*. 2003;42:1975–1981.
99. London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. *Hypertension*. 2001;38:434–438.
100. Jafar TH, Stark PC, Schmid CH, Landa M, Maschio G, de Jong PE, de Zeeuw D, Shahinfar S, Toto R, Levey AS. Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. *Ann Intern Med*. 2003;139:244–252.
101. Ibsen H, Olsen MH, Wachtell K, Borch-Johnsen K, Lindholm LH, Mogensen CE, Dahlöf B, Devereux RB, de Faire U, Fyhrquist F, Julius S, Kjeldsen SE, Lederballe-Pedersen O, Nieminen MS, Omvik P, Oparil S, Wan Y. Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: losartan intervention for endpoint reduction in hypertension study. *Hypertension*. 2005;45:198–202.
102. Wang TJ, Evans JC, Meigs JB, Rifai N, Fox CS, D'Agostino RB, Levy D, Vasani RS. Low-grade albuminuria and the risks of hypertension and blood pressure progression. *Circulation*. 2005;111:1370–1376.
103. Freedman BI, Langefeld CD, Lohman KK, Bowden DW, Carr JJ, Rich SS, Wagenknecht LE. Relationship between albuminuria and cardiovascular disease in type 2 diabetes. *J Am Soc Nephrol*. 2005;16:2156–2161.
104. Wenzel RR, Mann J, Jurgens C, Yildirim I, Bruck H, Philipp T, Mitchell A. The ETA-selective antagonist SPP301 on top of standard treatment reduces urinary albumin excretion rate in patients with diabetic nephropathy. *J Am Soc Nephrol*. 2005;16:58A.
105. Plato CF, Garvin JL. Nitric oxide, endothelin and nephron transport: potential interactions. *Clin Exp Pharmacol Physiol*. 1999;26:262–268.
106. Petrakopoulou P, Anthopoulos L, Muscholl M, Klauss V, von Scheidt W, Uberfuhr P, Meiser BM, Reichart B, Weis M. Coronary endothelial vasomotor function and vascular remodeling in heart transplant recipients randomized for tacrolimus or cyclosporine immunosuppression. *J Am Coll Cardiol*. 2006;47:1622–1629.
107. Silverborn M, Ambring A, Nilsson F, Friberg P, Jeppsson A. Blunted vascular response to endothelin-1 receptor blockade in cyclosporine-treated lung transplant recipients. *J Heart Lung Transplant*. 2005;24:665–670.
108. Cauduro RL, Costa C, Lhulier F, Garcia RG, Cabral RD, Goncalves LE, Manfro RC. Cyclosporine increases endothelin-1 plasma levels in renal transplant recipients. *Transplant Proc*. 2004;36:880–881.
109. Kon V, Hunley TE, Fogo A. Combined antagonism of endothelin A/B receptors links endothelin to vasoconstriction whereas angiotensin II effects fibrosis. Studies in chronic cyclosporine nephrotoxicity in rats. *Transplantation*. 1995;60:89–95.