# MINIREVIEW

# Endothelin-1 and Endothelin Receptor Antagonists in Cardiovascular Remodeling

## (44414)

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*Abstract.* **Endothelins build a peptide family composed of three isoforms, each of them containing 21 amino acids. Endothelin-1 is the isoform mainly responsible for any cardiovascular action and therefore the sole scope of this review. Endothelin-1 is the most potent endogenous vasoconstrictor known; in addition it acts as a potent (co)mitogen. There is a substantial body of experimental evidence that endothelin-1 may contribute not only to sustained vasoconstriction, but also to remodeling within the cardiovascular system. Thus, with the help of endothelin receptor antagonists (available for a few years) the involvement of mainly $ET_A$ receptors in structural diseases such as heart failure, pulmonary hypertension, atherosclerosis, restenosis, systemic hypertension, and chronic renal failure has been shown. These data make endothelin receptor antagonists, and especially those selective for the $ET_A$ receptor, promising agents for the treatment of chronic cardiovascular diseases associated with remodeling. Currently several chemically distinct, orally available members of this novel class of therapeutic agents are under clinical investigation.**

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Endothelin (ET) was described for the first time in 1988 as a "novel potent vasoconstrictor peptide produced by vascular endothelial cells" (1). Since then a huge body of work has emerged, characterizing three isoforms of endothelin and showing that primarily endothelin-1 (ET-1) is of importance in the mammalian cardiovascular system both as an autocrine and a paracrine hormone (2). There is currently much less knowledge about the function of the two other isoforms; however, endothelin-2 may function as a mediator (3) in the kidney, and some evidence points to a role of endothelin-3 as a mediator in the central nervous system and the intestinal tract (3). As the present review is solely concerned with chronic cardiovascular actions of endothelin it will focus exclusively on ET-1.

Up to now no intracellular storage for ET-1 has been identified. Increases in ET-1 levels are thought to be exclusively due to regulation on the level of gene transcription, with the *de novo* synthesis and release occurring in response to exogenous endothelial cell stimulation by a variety of mechanical and/or chemical stimuli. Factors promoting preproendothelin-1 synthesis include vasoactive hormones such as angiotensin II (4) or catecholamines (5), inflammatory mediators (6), and experimental conditions such as hypoxia (6) or shear stress (7). As has already been correctly described in the first publication on endothelin by Yanagisawa and colleagues (1), preproendothelin-1, a 212-amino acid peptide, is cleaved by a dibasic pair-specific endoprotease to big endothelin-1, composed of 38 amino acids. Big ET-1 is subsequently cleaved by one or more endothelin converting enzymes to the mature peptide, ET-1 (8, 9).

The effects of ET-1 are mediated *via* two different receptors, $ET_A$ and $ET_B$, which have both been cloned and pharmacologically characterized (10). On the vascular endothelium itself, only $ET_B$ receptors are to be found, mediating vasodilatation *via* stimulation of NO- and prostacyclin-release, ET-1 synthesis, and clearance of ET-1 from the circulation (11), as already mentioned. Thus the $ET_B$ receptor is probably not causally related to pathologies such as congestive heart failure (CHF), pulmonary hypertension, elevated systemic blood pressure, atherosclerosis, or renal

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failure. However, in some pathological situations such as coronary artery disease, an upregulation of $ET_B$ receptors was observed (12). The $ET_A$ receptor is located predominantly on vascular smooth muscle cells, but also on cardiomyocytes and fibroblasts. It is a classical heptahelical G-protein coupled receptor (10) activating phospholipase C to cause hydrolysis of phosphatidyl inositol and generation of cytosolic inositol triphosphate and membrane-bound diacylglycerol (2, 13). As reviewed recently for vascular smooth muscle cells by Force (14) and myocardial cells by Sugden and Bogoyevitch (13), the two most important groups of mitogenesis inducing factors, G-protein coupled receptors and growth factors, share several distal pathways. Most important among them are the c-Raf-1/ERK cascade and the activation of tyrosine kinases. However, much work remains to be done to clarify issues such as the role of transcription and translation factors in the development of hypertrophy and, given the similar distal pathways, what accounts for specific cellular responses to different growth factors and receptor-mediated stimuli such as ET-1. The mitogenic effect of ET-1 in vascular smooth muscle cells (15, 16) as well as in myocardial cells (13) is also primarily mediated *via* the $ET_A$ receptor. It has been shown that a direct correlation exists, at least *in vitro*, between $ET_A$ receptor density and ET-1 induced mitogenesis in human smooth muscle cells (17). There also exists a variable, but always small portion of $ET_B$ receptors on vascular smooth muscle cells which, if located, act *via* the same second messenger pathway as described for $ET_A$ receptors and serve the same function (2). As most of the mature ET-1 is secreted toward the abluminal side of the vascular wall, ET-1 acts primarily as an autocrine and paracrine hormone rather than an endocrine one (18, 19). Circulating ET-1 is thus considered a surrogate marker for elevated levels in the extracellular cleft (20); in addition it is rapidly cleared by the lung (11), thus having a half-life between 3 and 5 min. Nevertheless, by stimulating endothelial $ET_B$ receptors, the release of NO elevated circulating ET-1 levels could also be seen as a natural response to the overspill from elevated local levels, as NO is a potent inhibitor of ET-1 synthesis and actually downregulates ET-1 production on the gene level (21). For these reasons, experiments both in animals and volunteers to investigate the acute effects of exogenously administered high ET-1 concentrations either systemically (animal studies) or locally (forearm resistance experiments in man) are to be interpreted cautiously.

There is a growing body of evidence that ET-1, *via* stimulating collagen type IV synthesis in fibroblasts (22) has an important role in extracellular matrix formation, and thus also in remodeling especially after injuries within the cardiovascular system. Injuries initiating this process may be myocardial ischaemia, chronic vascular distensions due to pathologically elevated pressures both in the pulmonary as well as systemic arterial circulation, or local iatrogenic injury in the case of transluminal arterial revascularization. It recently has been shown in human cardiac fibroblasts that ET-1, *via* the $ET_A$ receptor, stimulates collagen synthesis without having a mitogenic effect on the fibroblasts themselves (23); this indicates an important role of ET-1 in the development of cardiac fibrosis and remodeling. Also in the kidney, extracellular matrix deposition and chronic functional deterioration into failure seems to be triggered, among other factors, by elevated protein excretion stimulating collagen synthesis *via* upregulation of the local ET-system (24).

From these introductory remarks it should become clear that ET-1 plays an important role in all chronic cardiovascular diseases associated with vasoconstriction, proliferation, and hypertrophy (Fig. 1). Over the last couple of years several pharmaceutical companies succeeded in identifying either selective $ET_A$ or balanced $ET_{A/B}$ receptor antagonists. An overview of the compounds currently most widely used either as a pharmaceutical tool or in clinical development is given in Table I. The usefulness as well as possible limitations of endothelin receptor antagonists in congestive heart failure (CHF), pulmonary hypertension, vascular hypertrophy associated with hypertension and artherosclerosis, restenosis, and late transplant atherosclerosis and rejection will be discussed in the following sections.

## Congestive Heart Failure (CHF)

The improvement of functional capacity as well as survival of patients with CHF by vasodilator therapy is generally accepted (25–27). Nevertheless still 60%–70% of CHF patients die within 5 years of diagnosis. This is probably due to incomplete normalization of vascular resistance with all

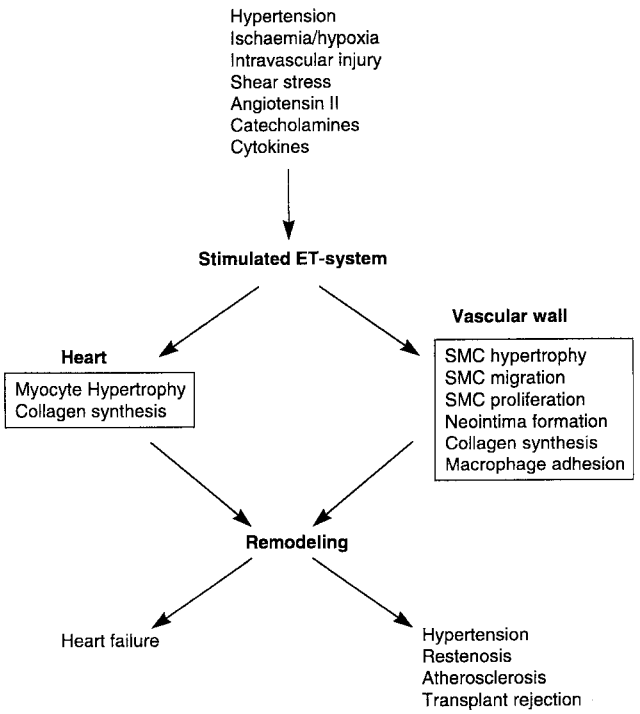


**Figure 1.** Involvement of a stimulated ET-1 system in cardiac and vascular remodeling.

**Table I.** Endothelin Receptor Antagonists Currently Being Used Experimentally or Clinically to Elucidate ET-1's Role in Chronic Cardiovascular Diseases

| Company | Compound/receptor | Proposed indications | Development status |
|---|---|---|---|
| Abbott | ABT 627 (=A127722)/$ET_A$ | Prostate cancer, CHF | Phase I/II |
| Banyu | BQ 123/$ET_A$ | | Pharmacological tool |
| Banyu | BQ 788/$ET_B$ | | Pharmacological tool |
| BMS | BMS 193884/$ET_A$ | CHF | Phase I/II |
| Knoll AG | LU 135252/$ET_A$ | CHF, Hypertension | Phase II |
| Merck KGaA | EMD 94246/$ET_A$ | CHF, Hypertension | Preclinical, discontinued |
| MSD | J-104132/$ET_A$ | Hypertension | Preclinical/Phase I |
| Parke-Davis | PD 156707/$ET_A$ | CV indications | Preclinical |
| Roche (Actelion) | Bosentan/$ET_{A/B}$ | CHF, Hypertension | Phase II/III |
| SKB | SB 209670/$ET_A$ | CV indications | Phase I/II |
| SKB | SB 217242/$ET_{A/B}$ | COPD | Phase I |
| Takeda | Tak 044/$ET_{A/B}$ | CAD, Renal failure | Phase II (peptidic, only iv) |
| Texas Biotech | TBC 11251/$ET_A$ | CHF, PPH | Phase I/II |
| Zeneca | ZD 1611/$ET_A$ | COPD, PPH | Preclinical/Phase I |

*Note.* CV = Cardiovascular; CAD = Coronary Artery Disease; CHF = Congestive Heart Failure; COPD = Chronic Obstructive Pulmonary Disease; PPH = Primary Pulmonary Hypertension

available therapies (25) or to their total lack of effect on secondary pulmonary hypertension, which more often than not accompanies severe CHF. ET-1 may be the culprit of pathologically increased vascular resistance both in the lung and in the arterial circulation. In addition ET-1 triggers intracellular events leading to long-term expression of fetal genes and protein synthesis, promoting growth and hypertrophy both in myocardium and in vascular smooth muscle (28, 29); thus, it may well be involved in the progressive vascular and cardiac remodeling seen in CHF. Elevated plasma and tissue ET-1 levels and changes in ET receptor density and activity have been demonstrated in CHF (for a recent review see Ref. 30). Thus antagonising ET-1 actions *via* $ET_A$ receptor blockade as a therapeutic target to improve functional capacity and survival in CHF patients seems well worth trying. Several animal models of heart failure have been published using ET receptor antagonists chronically.

**Left Ventricular Hypertrophy Induced by Hemodynamic Overload.** In rats with renal artery stenosis the effect of the $ET_A$ selective compound LU 127043 (31), on development of ventricular hypertrophy and blood pressure was (32) investigated either 2 or 10 days after surgery. In controls, plasma renin activity, left ventricular mass and left ventricular mRNA for β-myosin heavy chain were significantly elevated as early as 2 days after surgery. Blood pressure only started to rise on the third day and continued to increase until Day 10; also ventricular hypertrophy increased over time in the saline-treated rats. Despite the $ET_A$ receptor antagonist having no effect on plasma renin activity and blood pressure, it totally prevented left ventricular hypertrophy as well as re-expression of fetal phenotype mRNA at Day 2; this effect was still significant at Day 10, although to a somewhat lesser degree. The authors concluded that endothelin played a decisive role in early reprogramming and development of left ventricular hypertrophy in this model with elevated circulating angiotensin II levels whereas the later increase in hypertrophy, which was mediated *via* increased blood pressure, may be adaptive and not solely mediated *via* $ET_A$ receptors (32).

A similar result was found when hemodynamic overload was induced by aortic banding in rats (33). The selective $ET_A$ receptor blocker BQ 123, when given as a constant intravenous infusion, was able to block the myocardial hypertrophy 1 week after banding; however, after 2 weeks, no significant effect on hypertrophy was measurable. The authors also conclude that a stimulated endogenous endothelin system plays a role in the early initiation of the hypertrophic response in a setting of pressure overload (33), but loses some of its importance in chronic situations. At least part of the hypertrophy represents a "physiologic" response to sustained pressure overload.

**CHF After Coronary Occlusion in Rats.** The model most widely used over the last 15 years to evaluate experimentally the potential usefulness of drugs in human CHF has been published by Pfeffer's group (34). After left coronary artery occlusion, rats surviving the acute phase of myocardial infarction develop progressive left and right ventricular hypertrophy and remodeling. This model has been used by several groups to show beneficial effects of ACE-inhibitors which, primarily *via* reducing afterload, improve left ventricular hemodynamics, geometry, and ultimately survival in rats (reviewed in Ref. 35). More importantly, clinical trials using ACE inhibitors in CHF confirmed those beneficial effects in men (e.g., Ref. 27), thus showing the predictive value of this rat model for infarct-induced CHF in men.

The participation of ET-1 in the development of CHF and cardiac remodeling was shown in this model (36). It was seen that 3 weeks after myocardial infarction not only prepro-ET-1 mRNA levels as well as intramyocardial levels of mature ET-1 were significantly higher in the left ventricular myocardium, but also ET receptor density was elevated as compared to sham-operated controls (36). The model of infarction-induced CHF was used by several labo-

ratories to investigate the effects of ET receptor blockade. From the results available to date, two distinct sets of data need to be considered separately, data in animals treated from 7–10 days after myocardial infarction onward (late treatment) and those in animals treated immediately after experimental coronary occlusion (early treatment).

*Late treatment.* The first study showing significant positive effects was done by chronic infusion of BQ 123 ($ET_A$-selective receptor antagonist) over 12 weeks starting 10 days after acute myocardial infarction (37). It was seen that $ET_A$ receptor blockade was able to improve cardiac function by significantly reducing left ventricular enddiastolic pressure (LVEDP), right ventricular systolic pressure (RVSP) and central venous pressure compared to untreated control rats. Also cardiac structure was significantly improved by reducing left ventricular mass and cavity volume. These changes together resulted in a significantly lower mortality rate in treated rats as compared to untreated controls (37). The favorable effects of BQ 123 were attributed to blockade of $ET_A$ receptors whereby inhibiting the negative effect of elevated ET-1 levels on myocardial cells, regression of maladaptive cellular hypertrophic processes, reduction of myocardial contractility and significant reduction of afterload both in the systemic and pulmonary circulation, the latter effect described by the same group in more detail in a separate study (38). It was seen that from two weeks after ischaemia-induced CHF onwards significant pulmonary hypertension developed in rats, which was accompanied by a marked increase in pulmonary ET-1 production. These effects could significantly be reduced by chronic infusion of BQ 123 (38).

After the introduction of the orally available ET receptor antagonist LU 135252 ($ET_A$-selective) the effect of chronic oral treatment with 10 or 30 mg/kg/day over 10 weeks beginning 7 days after infarction on left ventricular remodeling and cardiac function was investigated (39). Systolic blood pressure and heart rate were measured in conscious animals; cardiac output and LV dimensions were measured ultrasonically in anesthetized rats followed by a complete hemodynamic profile. As compared to a placebo group, both doses of LU 135252 reduced systolic blood pressure, but only the higher dose reduced heart rate significantly. LU 135252 improved cardiac output and significantly reduced LV dilatation and deterioration of LV fractional shortening. LU 135252 also normalized LV enddiastolic as well as central venous pressures, but did not affect overall LV contractility. In infarct rats, collagen content in the portion of the left ventricular wall, not included in the scar area, was significantly higher, but normalized by treatment with this $ET_A$ receptor antagonist (39). Two differences to the study with BQ123 (37) are worth mentioning: the development of left ventricular hypertrophy was not reduced significantly (39), and, as there was no significant mortality in untreated controls over the same period, no differences in mortality could be described (see below).

The same group that conducted the study with LU 135252 also did a trial in rats treated (again starting 7 days after infarction) with the mixed $ET_{A/B}$ receptor antagonist bosentan for 9 months (40). A subgroup was sacrificed after 2 months for hemodynamic investigations. Bosentan reduced central venous pressure, LVEDP, LVSP and heart rate. At 2 months bosentan additionally reduced left ventricular dilatation and ventricular collagen content. After 9 months, the group treated with bosentan had a significantly higher survival rate than the controls (39), as was described for a selective $ET_A$ antagonist above (37). In an earlier study only the systemic hemodynamic effect of chronic oral bosentan in rats with established heart failure was studied over 6 weeks in conscious rats (41). A significant reduction in mean arterial pressure, which was additive to the effect of the ACE inhibitor cilazapril when administered concomitantly, was found (41).

*Early treatment.* In a study starting bosentan (100 mg/kg/day for 8 weeks) immediately on the day of experimental myocardial infarction (42), bosentan had no effect on elevated LVEDP. Right ventricular or pulmonary pressure parameters were not measured in this study. However, systemic blood pressure, heart rate, and ventricular ET-1 content were significantly reduced by treatment over 8 weeks. Also the rightward shift in the cardiac pressure volume curve, which was used in this study as an indicator of ventricular dilatation, was partially inhibited by treatment with the mixed $ET_{A/B}$ receptor antagonist (42). The effects were, overall, much less pronounced than in the studies in which treatment with an ET-receptor antagonist was initiated late after infarction. Even more pronounced differences to early treatment were found with some $ET_A$ selective receptor antagonists. When the compound EMD 94246 was given for 8 weeks starting on the day of acute myocardial infarction, no changes in left ventricular or systemic hemodynamic parameters were measurable compared to untreated CHF controls (43). The passive pressure volume curve was slightly, but significantly worse than in infarct controls, despite left ventricular volume and mass being unchanged. Right ventricular or pulmonary pressures were not measured (43). In a more elaborate study the effects of early treatment with the $ET_A$ selective receptor antagonist LU 135252 (and its racemic progenitor LU 127043) were investigated (44). Oral treatment was initiated within 24 hr after infarction and continued for 4 weeks. In this study treatment led to thinning of the left ventricular scar, further left ventricular dilatation, and an increase in LVEDP. However, despite this negative effect on LV function, $ET_A$ receptor blockade led to a significant improvement in RVSP, and right atrial pressure. Also the increase in pulmonary ET-1 content seen in controls was significantly reduced by LU 135252 (44). Similar results were found using the $ET_A$ selective antagonist ABT 627 from the first day of infarction onward; however, they have only been published as an abstract up to now (45).

With the possible exception of bosentan, which had a moderately positive effect on systemic hemodynamic pa-

rameters and no effect on LV cardiodynamics (42), the studies initiating ET receptor blockade early after infarction in the rat are pointing to a negative effect of early treatment. Unfortunately, only in two of the studies (44, 45) were right ventricular pressure parameters as well as scar thickness measured, so only these two studies will be considered for an explanatory discussion here. The detrimental effect of early ET receptor blockade on scar healing seems to lead to excessive LV dilatation and thus to inhibit any positive effect ET receptor antagonists would have when initiated later. The early and sustained use of selective $ET_A$ receptor antagonists was, however, still definitely positive in reducing pulmonary and RV pressures. The adverse effects of early $ET_A$ receptor blockade on scar healing and cardiac remodeling is in all likelihood due to the inhibition of collagen formation and thus wound healing and scar formation (see discussion of these effects at the beginning of this paper). As infarcts in the rat model are always huge (40%–50% of left ventricle) and always transmural, this negative effect of early treatment with ET receptor antagonists needs to be investigated carefully in additional species before conclusion for the clinical situation can be drawn. This becomes even more important in light of a considerable body of very positive data on ET antagonists in acute myocardial infarction. To name but a few it has been shown in pigs using both balanced (46) and selective $ET_A$ receptor antagonists (47) that infarct size after ischaemia and subsequent reperfusion is significantly reduced. This effect is most likely due to an inhibition of the no-reflow phenomenon by $ET_A$ receptor blockade, as has recently been shown even if the dogs were treated at the time of reperfusion only (48). In addition, a selective $ET_A$ blocker has recently been shown to inhibit leukocyte adhesion during reperfusion after global ischaemia in an *in vitro* model (49); if this effect is also seen *in vivo* its importance to reduce infarct size *via* inhibiting the early inflammatory response could be considerable. Appropriate experiments in different species should be conducted in the near future to settle the question if ET receptor antagonists should indeed not be initiated in the early phase after acute myocardial infarction. They then may lead to an impairment of scar healing that could induce detrimental left ventricular remodeling, counterbalancing their distinctly positive effects on afterload reduction and especially reduction of pressures in the pulmonary circulation.

**Pacing-Induced Heart Failure (Dilatative HF).** Overdrive pacing-induced HF is a model used in several species. After implantation of a cardiac pacemaker, the animals are constantly paced at about three to four times resting heart rate and thus develop a pronounced dilatative cardiomyopathy within 14 days. The effect of chronic $ET_A$ receptor blockade by oral treatment with 50 mg/kg/day LU 135252 on heart failure induced by rapid overdrive pacing in conscious mongrel dogs was studied. Dogs received either placebo or LU 135252 starting at Day 3 and continuing once daily for 2 weeks of pacing. All hemodynamic measurements were conducted with the pacemaker turned off (50). Compared to placebo, dogs treated with LU 135252 had a significantly smaller increase in left ventricular end-diastolic pressure as well as mean pulmonary artery pressure. Despite no changes in heart rate or arterial blood pressure, cardiac output was significantly increased in treated dogs with heart failure ($2.6 \pm 0.8$ vs. $1.9 \pm 0.6$ l/min). These data suggest that in pacing-induced heart failure, chronic $ET_A$ receptor blockade by LU 135252 improves hemodynamics *via* a vasodilator effect both on the pulmonary and the systemic vascular bed (50).

In a second study the effect of LU 135252 on cardiovascular and renal hemodynamics at two different time points was studied in a model of pacing-induced heart failure (51). Dogs were assigned randomly to four groups, and a possible dependence of the effect of $ET_A$ receptor blockade from the severity of CHF was investigated. At 10 days after pacing (mild CHF), no differences in cardiovascular or renal hemodynamics between control and treatment group could be detected. In contrast, after 21 days, LU 135252 treatment resulted in significantly higher cardiac output, augmented renal blood flow, and reduced renal vascular resistance. Systemic blood pressure remained unchanged. The authors conclude that ET-1 mediates renal and cardiovascular hemodynamic effects in severe CHF *via* the $ET_A$ receptor (51). The study supports a therapeutic potential for $ET_A$ receptor antagonism with LU 135252 by reversing systemic and renal vasoconstriction especially in severe heart failure.

Similar positive cardiodynamic effects were published by the same group using the $ET_A$ selective receptor antagonist A-127722 (52). However, no significant improvement in renal hemodynamics was seen with this compound, suggesting either differences between different antagonists in their action on the kidney or differences in the pharmacokinetic properties between different drugs.

In a rabbit model of chronic overdrive pacing-induced HF, the effect of the $ET_A$ receptor antagonist PD 156707 after chronic oral administration was investigated not only on global LV function but also on isolated myocyte function (53). Oral treatment with PD 156707 for 3 weeks improved LV fractional shortening and reduced the dimension of the end-diastolic left ventricle. These changes were not accompanied by a significant effect on systemic blood pressure. In myocytes isolated after 3 weeks of pacing, the shortening velocity was significantly reduced as compared to normal controls; this was normalized by concomitant treatment with the $ET_A$ receptor antagonist. The reduced responsiveness to adrenergic stimuli in myocytes from HF-controls was also normalized under treatment (53). Similarly positive results were published by the same group using pigs as an animal model and the $ET_A$ selective receptor antagonist BMS 193884 as a model drug (54). Some of the possible mechanisms discussed for these positive effects of $ET_A$ receptor blockade in this model of dilatative HF are a reduction of afterload by the drugs, increased myocardial blood flow under treatment, decreased pulmonary resistance and

thus an increased oxygenation capacity, and improved contractility of the myocytes due possibly to maintenance of calcium homeostasis.

**Short-Term Hemodynamic Effects of ET Receptor Antagonists in CHF.** Studies using the mixed $ET_{A/B}$ receptor antagonist acutely in established CHF were published in rats (55), dogs (56), and patients (57, 58). In rats with experimental CHF produced by an aorto-caval fistula, a significant improvement of regional renal blood flow was seen after bosentan. The findings indicate that ET-1 may be involved in the pathogenesis of renal hypoperfusion in severe decompensated CHF and that an ET receptor antagonist may have beneficial effects on renal hemodynamics (55). Similar effects were seen under chronic treatment with an $ET_A$ selective compound in a model of overdrive pacing-induced HF in dogs (51), as is discussed above. In acute heart failure induced by multiple intracoronary microembolizations in anesthetized dogs, intravenous bosentan was able to reduce systemic vascular resistance and improved overall LV performance (56), suggesting an involvement of ET-1 even in acute HF.

In the first clinical trial of an ET receptor antagonist in CHF published (57), intravenous infusion of the mixed $ET_{A/B}$ receptor antagonist bosentan was studied in patients withdrawn from ACE inhibitors for four half-lives prior to the study. Bosentan reduced systemic and pulmonary pressures without inducing an increase in heart rate and significantly increased cardiac output (57). In a second study with bosentan, which was conducted in a randomized, placebo-controlled manner over 14 days on top of standard treatment including ACE inhibitors, it could be seen that the beneficial effects of ET receptor blockade occurred additively to the one of ACE-inhibition (58). Long-term trials are currently being conducted with several compounds. They will show if ET receptor antagonists will be able to not only acutely or subacutely improve hemodynamic parameters, but also have a significant impact on ventricular remodeling (as has been discussed for animal experiments) and thus will reduce morbidity and mortality in addition to chronic ACE-inhibitor treatment.

## Pulmonary Hypertension

Pulmonary hypertension is associated clinically with a variety of diseases ranging from primary pulmonary hypertension to hypoxic lung disease, congenital heart diseases, and CHF (see above). All those forms of pulmonary hypertension have, especially in the later stages, common features like pulmonary arterial vasoconstriction and vascular remodeling due to smooth muscle cell proliferation and extracellular matrix expansion. As in CHF, also in pulmonary hypertension, ET-1 has been shown to be causally involved in human pulmonary vasculature (59). As has been discussed in the previous section, secondary pulmonary hypertension as well as right ventricular remodeling induced by CHF is effectively reduced by chronic ET receptor antagonist treatment.

**Pulmonary Hypertension Induced by Monocrotaline.** Monocrotaline is a toxic alkaloid inducing pulmonary vascular endothelial damage and is thus used to induce primary pulmonary hypertension in animal models (60). The development of pulmonary hypertension was shown to be associated with elevated ET-1 levels (61), and BQ 123 was used to study possible effects of $ET_A$ receptor blockade in the same study (61). Continuous intravenous infusion of BQ 123 was able to reduce the development of pulmonary hypertension as well as right ventricular hypertrophy. Oral treatment with the $ET_A$ selective receptor antagonist LU 135252 for 3 weeks not only led to significant reduction in elevated pulmonary artery pressure (Fig. 2), but also to a significant decrease in right ventricular hypertrophy (61). *In vitro* studies at the end of the 3-week treatment period showed a marked improvement of NO-dependent pulmonary artery vasodilatation as compared to untreated controls (62). In contrast, chronic treatment during evolving pulmonary hypertension with the mixed $ET_{A/B}$ receptor antagonist bosentan was not able to prevent either pulmonary hypertension or right ventricular hypertrophy (63). This difference suggests that simultaneous blockage of $ET_A$ and $ET_B$ receptors may counteract the positive effects of selective $ET_A$ blockade; possibly by interfering with the pulmonary ET-1 clearance as well as the NO-mediated vasodilatation mediated *via* $ET_B$ receptor stimulation, as discussed above. A negative effect of $ET_B$ receptor blockade was also seen in dogs with monocrotaline-induced pulmonary hypertension persisting for 8 weeks, if either a selective $ET_A$ or a selective $ET_B$ receptor antagonist (FR139317 and RES-701–1, resp.) were infused acutely into the right atrium (64). Acute $ET_A$ blockade reduced pulmonary vascular resistance and mean pulmonary artery pressure, whereas $ET_B$ blockade with RES-701–1 further increased these parameters. More importantly, in a recent paper it was seen that onset of

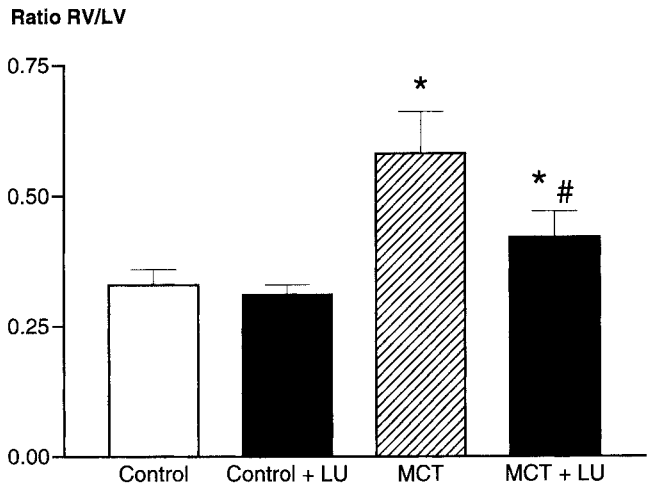


**Figure 2.** Effect of 2 weeks of oral treatment with 50 mg/kg/day LU 135252 on right ventricular hypertrophy expressed as a ratio of right ventricular (RV) to left ventricular (LV) mass. MCT = monocrotaline; mean ± SEM; $n$ = 15–18/group; * = $P < 0.05$ vs. control, # = $P < 0.05$ vs. MCT (adapted from Ref. 62).

treatment with the $ET_A$ receptor antagonist LU 135252 commencing 2 weeks after established severe pulmonary hypertension in the rat model was able to increase the survival rate and to significantly decrease right ventricular pressure and hypertrophy (65). Despite treatment not being able to reverse the pulmonary hypertension it could significantly prevent further deterioration and additionally improve pulmonary artery compliance.

**Hypoxia Induced Pulmonary Hypertension.** The possible involvement of ET-1 and its receptors in increased vascular tone in chronic hypoxic pulmonary hypertension was investigated in pulmonary arteries isolated from rats previously exposed to 2 weeks of low oxygen(10%) and compared to vessels from rats kept in normoxic conditions (66). ET-1 in both groups induced a marked pulmonary artery vasoconstriction that was, however, more potent in vessels from hypoxic rats. In both groups, addition of the $ET_A$ receptor antagonist FR 139317 could antagonize ET-1's vasoconstrictory effect. However, only in hypoxic conditions was the $ET_B$ selective agonist sarafotoxin S6c able to induce a small vasoconstriction, thus pointing to an additional role of those receptors in chronic hypoxic induced pulmonary hypertension (66). In line with this *in vitro* result are data showing a positive effect of the mixed $ET_{A/B}$ receptor antagonist bosentan, which in this model (as opposed to the monocrotaline model discussed above) was able to largely prevent the effect of chronic hypoxia to increase vascular wall thickness of small arteries as well as the right ventricular hypertrophy and the elevated pulmonary pressures (67). In a second study, however, the effects reported to be achievable by bosentan treatment were less pronounced (68). Chronic treatment with $ET_A$ selective receptor antagonists were consistently reported to have positive effects in chronic hypoxia–induced pulmonary hypertension and right ventricular as well as pulmonary vascular hypertrophy when given in parallel to the evolution of the disease (69, 70). More importantly, it was shown that treatment with both bosentan (67) and the $ET_A$-selective compound A-127722 (69) were able to at least partially reverse established pulmonary hypertension and pulmonary vascular hypertrophy despite persisting hypoxia when treatment was started only 2 weeks after establishing pulmonary hypertension. Neither treatment reversed right ventricular hypertrophy, most likely because elevated pressure was not normalized but reduced by about 50% only. Thus, as in the case of pressure overload induced left ventricular hypertrophy discussed above, this hypertrophic response may well be a chronic adaptation to elevated pressures in the respective systems.

## Hypertension and Vascular Hypertrophy

The role of endogenous ET-1 in systemic hypertension has recently been reviewed in two excellent papers (71, 72) and will only be discussed here in connection with vascular remodeling. As pointed out in the two reviews mentioned, ET-1 seems not to be involved in all forms of human and experimental hypertension, but gains more and more importance with increasing severity of hypertension as well as in all forms associated with salt sensitivity. Thus certain populations, such as African-Americans, have an activated ET system (73) and may thus benefit more from ET receptor antagonist treatment than hypertensives not being salt-sensitive. Nevertheless, with hypertension typically persisting over many years the remodeling and antiatherosclerotic actions of ET antagonists, which will be reviewed briefly below, are likely to become more and more important, as ET receptor antagonists are likely to have considerable potential in protecting end-organs from the deleterious consequences of chronically elevated blood pressure. It has recently been described that in resistance arteries from patients suffering from severe chronic hypertension, the ET-1 gene expression was significantly enhanced as compared to normotensive or only mildly hypertensive patients (74). As there exists a huge body of literature on these topics (see Refs. 71, 72) the authors are concentrating in this section on results derived from experiments mostly connected to work done with LU 135252.

**Effect on Blood Pressure and Arterial Remodeling in Different Hypertensive Rat Models.** The effect of chronic $ET_A$ receptor blockade by LU 135252 or A 127722 was investigated in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, spontaneously hypertensive rats (SHR), and one-kidney-one-clip Goldblatt(1K1C) hypertensive rats (75). Systolic blood pressure, as measured by tail cuff plethysmography, was lowered moderately but significantly in DOCA-salt hypertensive rats, but unaffected by $ET_A$ receptor antagonism both in SHR and 1K1C rats. It was concluded by the authors that systolic blood pressure was lowered only in the model that is known to chronically over-express ET-1 (76), but not in the two other models that do not.

In a second study the effect of the $ET_A$-selective endothelin antagonist LU 135252 on blood pressure was tested with telemetric recording methods in conscious unrestrained spontaneously hypertensive rats. There were significant reductions in blood pressure sustained over 24-hrs at the end of a 2-week treatment period already at low doses of the $ET_A$ receptor antagonist (Fig. 3)(Becker R, personal communication). Thus it may well be that using a more sensitive method like intra-arterial measurement of blood pressure is unmasking a pressure response not seen under the stressful conditions of tail cuff measurement.

In another study the effect of a 2-week treatment with LU 135252 on the dilator response of small arteries of normotensive WKY or hypertensive SHR rats was investigated (77). Whereas $ET_A$ blockade again had no significant effect on elevated blood pressure in SHR, treatment improved flow-related dilatation of small mesenteric arteries by 73% as compared to untreated SHR. There was no change in WKY. Acetylcholine-induced dilatation was also significantly improved by LU 135252, whereas endothelium-

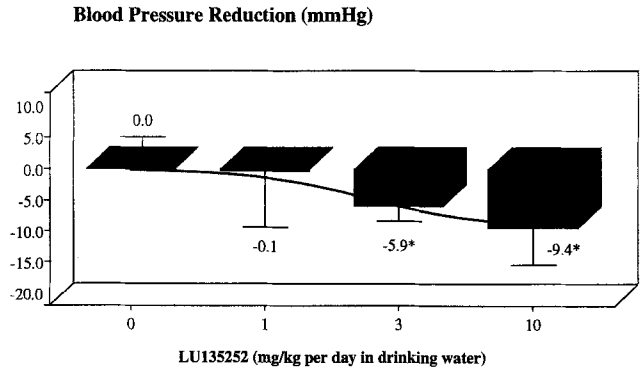


**Figure 3.** Blood pressure effects of chronic oral $ET_A$ receptor blockade in spontaneously hypertensive rats. Bars represent telemetrically measured mean arterial blood pressure reductions over 24 hr at the end of a 2-week treatment period with LU 135252. Mean ± SD; * = $P < 0.05$ vs. controls (Becker R, personal communication)

independent effects were not changed (77); both effects indicated a preservation of endothelial function in small arteries despite the persistence of hypertension. Furthermore, aortic hypertrophy was totally normalized by the compounds. DOCA salt rats had more apoptotic cells in the aortic wall than normal control rats. This already increased apoptotic rate was additionally significantly increased by chronic $ET_A$ receptor antagonism. The authors conclude that $ET_A$ receptor antagonists may act in part by accentuating apoptosis, thereby normalizing vascular hypertrophy and improving elasticity of the arteries (78). This effect could contribute to the antihypertensive effect and to reduction in end-organ damage.

To study the involvement of ET-1 in another model of salt-induced hypertension, Dahl rats received 4% NaCl alone or in combination with LU 135252 for 8 weeks (79). In addition to blood pressure measurements, the geometry and reactivity of small mesenteric and basilar arteries were studied *in vitro*. Chronic salt administration increased systolic blood pressure significantly by 37 ± 3 mmHg as well as the media/lumen ratio in these small arteries. Endothelium-dependent relaxations to acetylcholine as well as contractions to ET-1 were impaired in mesenteric arteries of salt-sensitive rats on high NaCl diet. Treatment with an $ET_A$ receptor antagonist was able to prevent part of the blood pressure increase, as well as structural and functional alterations (79).

In the same study, endothelium-dependent and independent renal arterial function was investigated (Barton M, personal communication). High sodium diet increased blood pressure, renal arterial tissue weight as well as vascular ET-1 content. Chronic treatment with LU 135252 normalized changes in tissue weight and ET-1 content. Endothelium-dependent contractions to acetylcholine, increased more than 3-fold with a high-sodium diet, were correlated with tissue ET-1 levels and normalized by $ET_A$ receptor blockade (Barton M, personal communication).

The effect of $ET_A$ receptor antagonism on vascular ET-1 content, aortic hypertrophy, and aortic reactivity *in vitro* was studied (80). Aortic ET-1 content in Dahl salt-sensitive control rats was 4.2 times higher than in salt-resistant controls; aortic hypertrophy was also significantly more pronounced. In addition, sodium diet reduced endothelium-dependent relaxations to acetylcholine as well as contractions to ET-1. Chronic treatment with LU 135252 normalized sodium-induced changes in vascular reactivity, tissue ET-1 protein content, and aortic structure, thus again pointing to potential therapeutic benefit in hypertension associated with structural vascular changes (80). The mechanisms by which $ET_A$ receptor blockade prevents the increase in vascular ET-1 content *in vivo* are currently unclear; however, they may involve autocrine, $ET_A$ receptor–mediated regulation of ET-1 mRNA and protein as has been observed in vascular smooth muscle (81) and cardiac fibroblasts (82). Furthermore, the reduction of ET-1 tissue levels could be related to LU 135252-mediated restoration of endothelial release of NO, a potent inhibitor of ET-1 production (83). However, in chronic NO-deficiency, ET-1 seems to play a minor role in both blood pressure development as well as vascular hypertrophy. Three independent studies using either the mixed $ET_{A/B}$ receptor blocker bosentan over 2 weeks (84, 85) or the $ET_A$ selective compound A-127722 (86) were not able to show significant effects of ET receptor blockade.

Studies in rats were carried out to determine if elevated angiotensin II mediated hypertension, vascular hypertrophy, and renal ET-1 content *via* $ET_A$ receptors (87–89). Wistar Kyoto rats were subjected for 2 weeks to angiotensin II infusions (200 ng/kg/min) alone or in combination with LU 135252. Ang II increased tail cuff pressure by 35 ± 5 mmHg as compared to controls. This effect was significantly reduced in the LU 135252- treated group (17 ± 4 mmHg). LU 135252 by itself had shown no effect on normal blood pressure. Ang II produced pronounced vascular hypertrophy as assessed by cross-sectional area both in mesenteric and basilar arteries at the end of the 2-week period. $ET_A$ receptor antagonism completely prevented vascular hypertrophy in both vascular beds. These results suggest that part of the proliferative effects of Ang II *in vivo* are mediated by ET-1 acting on $ET_A$ receptors (87). Whether the newly described dual receptor for ET-1 and Ang II (90) exists in vascular smooth muscle and if yes, if it is accessible by antagonists specifically designed for the $ET_A$ receptor, such as LU 135252, needs to be investigated.

The functional integrity of isolated aortic rings from Ang II-infused animals was studied (88). It was seen that Ang II infusion significantly reduced endothelium-dependent relaxations induced by acetylcholine. Treatment with LU 135252 almost normalized endothelial function. The $ET_A$ receptor antagonist had no effect on endothelium-independent relaxation to sodium nitroprusside, which was also impaired by Ang II. Contractions to endothelin-1 and norepinephrine were also impaired by Ang II and restored by concomitant chronic treatment with LU 135252 (88). Similar results were seen with another selective $ET_A$ recep-

tor antagonist, PD155080 (91). Ang II for 2 weeks increased ET-1 protein content in the aorta (4.7-fold) and femoral artery (1.6-fold) as well as in kidneys (3-fold) over WKY controls. Functional tissue endothelin–converting enzyme (ECE) activity was significantly increased. The Ang II-induced increases in tissue ET-1 content and functional ECE activity were completely normalized by treatment with LU 135252 over 2 weeks (89). Additional data on the effect of Ang II-induced tissue uptake of ET-1 show that ET uptake is only increased in kidneys, but not in the myocardium (92). This increased ET uptake could be totally normalized by chronic oral treatment with LU 135252. These data suggest that the kidney, but not the myocardium, is a target for Ang II-mediated activation of the endothelin system.

## Restenosis and Atherosclerosis

**Restenosis.** The role of endothelin as well as the effects of endothelin receptor antagonists in various animal models of restenosis have very recently been reviewed by the authors of this present review (93). Briefly, there is evidence that ET-1 augments and $ET_A$ receptor antagonists attenuate the degree of media hypertrophy and neointima formation following balloon angioplasty in carotid arteries of rats (94–96), iliac arteries of pigs (97), as well as coronary arteries of pigs (93, 98). These results identify local sites of ET-1 production and neighboring receptors where this peptide may be involved in neointima formation following this procedure. In addition, locally elevated ET-1 levels, which were found in human coronary artery plaques (99) as well as in the coronary sinus immediately after PTCA (100), act as a chemoattractant for monocytes and macrophages and, *via* stimulation of fibroblast $ET_A$ receptors, lead to matrix expansion by enhancing collagen production. From the experimental studies available to date it becomes likely that elevated endogenous ET-1, acting *via* $ET_A$ receptors, is not only involved in initial smooth muscle cell migration and proliferation and thus neointima formation, but also in excessive collagen production and accumulation of extracellular matrix in later stages of the healing process. $ET_A$ receptor antagonists may thus, for the first time, provide a pharmacological means to reduce recoil late after PTCA.

**Anti-Atherosclerotic Effects.** Over the last few years evidence was gained to underline the importance of a stimulated local ET system in the development of atherosclerosis. In native human vessel atherosclerosis it has been found that, in addition to endothelial cells, macrophages and smooth muscle cells are also immunoreactive toward ET-1 (99). In addition, patients with mild atherosclerosis, who during diagnostic coronary artery angiography reacted to an intracoronary acetycholine injection with vasoconstriction, had significantly higher coronary sinus ET-1 levels than patients having a normal vasodilatory response (101). Similar results, correlating increased tissue immunoreactivity in specimen from human aorta to elevated circulating ET-1 levels in severely atherosclerotic patients have been published (102). The same group was able to show in experimentally hypercholesteraemic pigs that both arterial smooth muscle cell immunoreactivity to ET-1 as well as circulating systemic ET-1 levels were significantly higher than in normal controls (103). In cultured human endothelial cells as well as intact porcine blood vessel specimen *in vitro,* it was found that oxidized low-density lipoproteins induce mRNA expression as well as release of ET-1 (104). This suggests that oxidized LDL may be a mediator of increased local ET-1 production in atherosclerosis and thus one of the initiating events of macrophage adhesion, smooth muscle cell proliferation, and progression of atherosclerotic disease. In a model of hypercholesterolemia-induced atherosclerosis in cholesterol-fed hamsters, the chronic treatment with the selective $ET_A$ receptor antagonist BMS-182874 significantly inhibited fatty streak formation (105).

ApoE knockout mice, when fed with a physiological high-fat diet, develop pronounced atherosclerotic lesions in the aorta. Chronic treatment with the $ET_A$ receptor antagonist LU 135252 reduced the percentage of lesions measurable in the aorta significantly; plasma cholesterol levels were not reduced (106). Endothelial function measured *in vitro* at the end of the experiments was almost completely restored (106). Maximal relaxation to acetylcholine was improved from 56% ± 4% in untreated controls to 93% ± 2% after chronic LU 135252 treatment. These data suggest a causal role of endothelin in the development of atherosclerosis and the concomitant endothelial dysfunction.

Another genetic model, the LDL receptor–deficient mouse, is considered a relevant model for hereditary familial hypercholesterimia (107). These mice were fed with a high-cholesterol diet, and chronically treated with the balanced $ET_{A/B}$ receptor antagonist LU 224332. LU 224332 significantly reduced aortic atherosclerotic lesions; general clinical signs, like xantholasma and limb swelling, were also improved in these animals.

## Transplant Atherosclerosis and Late Transplant Rejection

The success of allograft transplantation in human medicine, especially in renal and cardiac transplantation, is impressive. Nevertheless, long-term allograft survival is still limited due to a pronounced vasculopathy, called allograft atherosclerosis, both in hearts and kidneys. In heart allografts, more than 40% of the patients suffer from pronounced and generalized atherosclerosis 5 years after transplantation, and this vascular remodeling is the main cause for recurrent myocardial infarctions, leading to malignant arrhythmias, CHF, and death (108). In the renal allografts in addition to graft atherosclerosis, pronounced extracellular matrix deposition occurs, in addition to a limiting of renal function and an increase in protein excretion (24).

**Cardiac Transplantation.** In human coronary arteries harvested from patients who, due to pronounced allograft atherosclerosis were subjected to a second heart transplantation, were studied and compared to control coronary

arteries from hearts being explanted from patients suffering idiopathic dilatative cardiomyopathy without apparent atherosclerosis (108).

In 85% of the patients suffering from graft atherosclerosis, intense ET-1 immunostaining was found localized mainly in vascular smooth muscle cells, but also in macrophages and endothelial cells. ET-1 was more frequently present in lipid-rich, atheromatous regions than in lipid-poor lesions. In contrast, nonatherosclerotic arteries from hearts suffering dilatative cardiomyopathies had almost no ET-1 immunostaining (108). This study for the first time showed strong evidence for the importance of ET-1 in the pathogenesis of cardiac graft atherosclerosis. In animal experiments, allograft rejection in rats was also correlated to accelerated transplant atherosclerosis and highly elevated ET-1 immunostaining (109, 110). Recently, the role of chronic oral treatment with the mixed $ET_{A/B}$ receptor antagonist bosentan was described in a rat model of chronic cardiac allograft atherosclerosis (111). In this study, Lewis rats received either heterotopic hearts either from Lewis or from Brown-Norway donor rats; the latter were randomized into two groups, one of which received bosentan. At the histologic examination at the end of the study, it was seen that bosentan treatment was able to reduce atherosclerotic lesions in Brown-Norway hearts to levels similar to those in Lewis to Lewis transplantations (Fig. 4). In addition, tissue ET-1 immunoreactivity, which was significantly elevated in untreated controls, was reduced (111). Oral treatment with the mixed $ET_{A/B}$ receptor antagonist was thus able to significantly reduce graft atherosclerosis in this model.

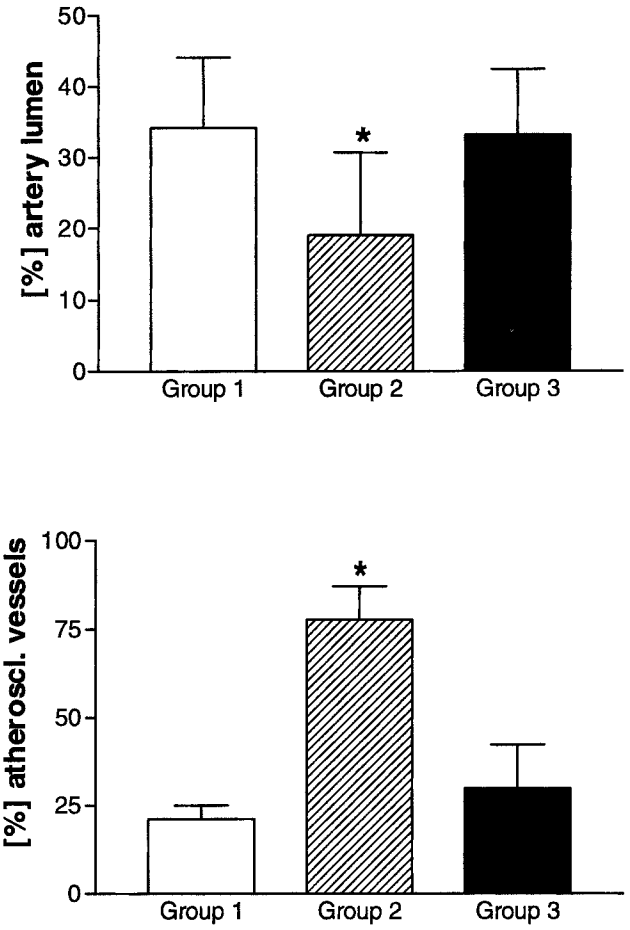


**Figure 4.** Graft atherosclerosis in rat hearts 120 days after heterotopic transplantation and the effect of oral treatment with the balanced $ET_{A/B}$ receptor antagonist bosentan. Group 1: Heterotopic hearts Lewis to Lewis rats; Group 2: Heterotopic hearts Brown Norway to Lewis rats, control; Group 3: Heterotopic hearts Brown Norway to Lewis rats, 20 mg/kg/day bosentan; mean ± SD; * = $P < 0.05$ vs. Group 1 (adapted from Ref. 111).

**Renal Transplantation.** In addition to its well-established role in postischaemic acute renal failure (recently reviewed in Ref. 112) the ET system might also be involved in the pathogenesis of post-transplant acute tubular necrosis and delayed graft function in the renal transplant setting. The underlying ischaemic lesions arising from explantation and organ harvesting during "cold ischaemia" share similarities with "warm ischaemia" during postischaemic acute renal failure. It was shown that in initially ischaemic kidneys, ET-1 accumulates during cold storage of organs and was also detectable in the effluent preservation solution (113). Furthermore it has been demonstrated that flushing renal allografts with cold Ringer's lactate containing an $ET_A$ receptor antagonist attenuated reperfusion injury in a rat model of kidney transplantation (114).

Chronic renal transplant rejection is the most common cause of graft loss in transplantation medicine. Up to now no treatment strategy has been successful, as the pathomechanism of chronic rejection is still poorly understood. In the well-established model of chronic renal rejection in the Fisher-to-Lewis rat model, the influence of LU 135252 was investigated in a long-term trial (115–117). Chronic oral treatment with LU 135252 improved allograft survival dramatically. At 24 weeks, when the study was terminated, only 38% of the control animals were alive, whereas in the treated group 92% of the animals still lived. This increase in survival was associated with improved renal function, less morphological changes, and a significant reduction in cell surface markers of inflammation. In addition a markedly reduced allograft infiltration by monocytes/macrophages was observed in the group treated chronically with LU 135252. Also activation parameters, such as MHC-II expression and T-cell infiltration were significantly reduced (115–117). The data demonstrate an important role for $ET_A$ receptor blockade in chronic transplant rejection and are likely to have a major clinical impact, especially as a recent study showed the major involvement of a stimulated ET-1 system in chronic allograft rejection in men (118). In patients with chronic renal rejection and transplant atherosclerosis and glomerulosclerosis, vascular ET-1 expression was more than six times higher than in normal kidneys or in kidneys from patients suffering from acute rejection. It was also demonstrated that macrophage-associated cytokines stimulated ET-1 secretion in human endothelial cells, vascular smooth muscle, and mesangial cells. These clinical results demonstrated for the first time that a locally stimu-

lated ET-1 system was causally related to chronic renal transplant rejection in man (118).

**Future Outlook and Clinical Perspectives** Since the discovery of the endothelin peptide family in 1988 our knowledge of their role in the pathophysiology of many disease states has profoundly increased by numerous *in vitro* and *in vivo* studies worldwide. This holds particularly true for the importance of the ET-1 isoform in chronic cardiovascular pathologies associated with remodeling processes alluded to in this review. Most of the results were derived from investigating the effect of several ET receptor antagonists made available over the last few years. Nevertheless, the transfer of data from experimental pharmacology to clinical practice, which is now under way, is necessary to establish the possible value of this new therapeutic principle. Particularly intriguing is the use of ET receptor antagonists in cardiovascular and also renal diseases characterized not only by sustained vasoconstriction but also by being additionally associated with atherosclerotic or hypertrophic processes like chronic heart failure, pulmonary hypertension, chronic renal failure, or atherosclerosis and restenosis. In appropriate animal models mimicking these disease states ET receptor antagonists have significantly improved long-term outcome. The authors are aware that there are not only differences between species and organs within each species with regard to ET receptor subtype density and distribution, but also between different pathological models. However, from the limited amount of data available from healthy as well as pathological human tissue, it seems valid to conclude that $ET_A$ receptors are by far dominant over $ET_B$ receptors in any investigated tissue derived from the human cardiovascular system (e.g., Ref. 2). In addition, stimulation of $ET_B$ receptors located on the vascular endothelium by ET-1 may well contribute to many positive actions of $ET_A$ receptor blockade, as ET-1's vasodilating and antiproliferative actions are regulated by endothelial $ET_B$ receptor–mediated NO-release. These positive effects may indeed be partially masked by the vasoconstrictive and mitogenic effect mediated *via* the more dominant $ET_A$ receptors. ET-1 could thus exert its positive cardiovascular effects and contribute to the $ET_A$ receptor antagonists therapeutic usefulness if such an $ET_A$ selective compound were used chronically. As several long-term, large-scale clinical trials with different compounds are either currently being conducted or planned, the questions raised will be solved within the next few years, adding new, highly effective drugs to reduce cardiovascular morbidity and mortality further, which will benefit chronically and severely ill patients.


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