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Endothelin receptor antagonists influence cardiovascular morphology in uremic rats

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Background. It is generally held that renal failure results in blood pressure (BP)-independent structural changes of the myocardium and the vasculature. The contribution, if any, of endothelin (ET) to these changes has been unknown.

Methods. We morphometrically studied random samples of the left ventricle myocardium and small intramyocardial arteries in subtotally (5/6) nephrectomized (SNx) male Sprague-Dawley rats treated with either the selective ET_A receptor antagonist BMS182874 (30 mg/kg/day) or the nonselective ET_A/ET_B receptor antagonist Ro46-2005 (30 mg/kg/day) in comparison with either sham-operated rats, untreated SNx, or SNx rats treated with the angiotensin-converting enzyme inhibitor trandolapril (0.1 mg/kg/day).

Results. Eight weeks later, systolic BP was lower in trandolapril-treated SNx compared with untreated SNx animals. No decrease in BP was seen following either ET receptor antagonist at the dose used. A significantly increased volume density of the myocardial interstitium was found in untreated SNx rats as compared with sham-operated controls. Such interstitial expansion was prevented by trandolapril and either ET receptor antagonist. SNx caused a substantial increase in the wall thickness of small intramyocardial arteries. The increase was prevented by trandolapril or BMS182874 treatment. The arteriolar wall:lumen ratio was significantly lower in all treated groups when compared with untreated SNx. In contrast, only trandolapril, but not the ET receptor antagonists, attenuated thickening of the aortic media in SNx animals.

Conclusions. The ET_A-selective and ET_A/ET_B-nonselective receptor antagonists appear to prevent development of myocardial fibrosis and structural changes of small intramyocardial arteries in experimental chronic renal failure. This effect is independent of systemic BP.

Cardiovascular abnormalities are known to be the leading cause of morbidity and mortality in uremic patients [1, 2]. Structural abnormalities of the cardiovascular system, for example, wall thickening of intramyocardial and peripheral arteries, as well as increased myocardial fibrosis, have been documented in rats with experimental chronic renal failure [3–6] and in patients with end-stage renal disease [7]. These changes may have important functional consequences, such as reduced coronary reserve, reduced vascular and left ventricular compliance, and increased mean arterial blood pressure (BP) [8–10]. It is of note that the structural changes are, at least in part, independent of the level of systemic BP [3–5, 11].

There is accumulating evidence that endothelin (ET), a potent endogenous vasodilator and mitogenic agent, is involved in chronic progressive renal failure [12–15] and in cardiovascular disease [16–18]. Expression of all three ET isopeptides, predominantly ET-1, has been demonstrated in the mammalian heart, particularly in cardiomyocytes [19, 20] and in the endocardium [21]. ET-1 is the only ET isopeptide that is generated in the endothelium and vascular smooth muscle cells (VSMCs) [22, 23]. Both the ET_A and ET_B receptor subtypes were identified in the myocardium and endocardium as well as in VSMCs [24, 25]. The ET_A receptors account for approximately 80% of the total cardiac ET receptor pool [26]. Nevertheless, ET_B receptors have been reported to be the major subtype in myocardial capillaries [27]. A role of ET has been postulated in the pathogenesis of malignant hypertension [16, 28], coronary artery disease [17, 29], and congestive heart failure [18, 30]. Its contribution, if any, to the development of myocardiopathy and vascular remodeling in renal failure remains obscure.

In this article, we selected the remnant kidney model of renal failure and investigated the effect of ET_A-selective and ET_A/ET_B-nonselective receptor antagonists on the structure of myocardium and of arterial vessels, both of the muscular and elastic type. A particular effort was made to examine whether a potential effect was dependent on changes in systemic BP and in left ventricular mass.

Key words: myocardial arteries, fibrosis, renal failure, subtotal nephrectomy, uremia, blood pressure, hypertension.

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METHODS

Animals and experimental design

Male Sprague-Dawley rats weighing 200 to 230 g were fed a standard rat chow (19% protein, 0.25% sodium), with free access to tap water. The animals were allotted to five groups. The animals of group 1 ($N = 8$) were sham-operated (decapsulation of the kidneys) and were further left untreated. In groups 2 to 5, 5/6 subtotal nephrectomy (SNx) was performed under isofluran inhalation anesthesia. After ablation of the right kidney, a week later, both poles of the left kidney were resected. Following the operation, the animals of group 2 ($N = 8$) received no treatment. As a positive control, group 3 ($N = 8$) received the angiotensin (Ang)-converting enzyme (ACE) inhibitor trandolapril (Knoll, Ludwigshafen, Germany) by gavage at a dose 0.1 mg/kg/day. Group 4 ($N = 8$) was treated with the orally active nonpeptide ET_A receptor antagonist BMS182874 (Bristol-Myers Squibb, Princeton, NJ, USA) at a dose of 30 mg/kg/day. Group 5 ($N = 10$) was treated with the orally active nonpeptide ET_A/ET_B-nonselective receptor antagonist Ro46–2005 (Hoffman-La Roche, Basel, Switzerland) at a dose of 30 mg/kg/day. The treatments in groups 3, 4, and 5 continued over eight weeks. Body weight, systolic BP measured by tail plethysmography, hematocrit, serum and urinary creatinine, and plasma renin activity were determined before surgery, four weeks later, and at the end of the experiment. Serum and urinary creatinine were measured using the Jaffé reaction. Plasma renin activity was determined using a radioimmunoassay for angiotensin I (Ang I).

Morphological techniques

The experiment was terminated by retrograde perfusion of the aldehyde fixative via the abdominal aorta as described in detail elsewhere [31]. The heart and aorta were excised, with the left ventricle separated, weighed, and sliced perpendicular to the longitudinal axis. Two slices of each left ventricle were randomly sampled and sectioned for stereologic investigation using the orientator technique as described [32]. The resulting eight left myocardial pieces per animal were embedded in Epon-Araldite, and semithin (0.5 m) isotropic uniform random sections were made to be further stained with methylene blue and basic fuchsin. The sections were examined by light microscopy with oil immersion and phase contrast at a magnification ×1,000. Volume density of the myocardial interstitium was measured in each section using the point counting method and was averaged for each animal. Wall thickness and lumen diameter of small (30 to 70 m in diameter) intramyocardial arteries were assessed by planimetry using a semiautomatic image analysis system (IBAS2; Contron, Eding, Germany). Arteries were defined as vessels having a complete layer of smooth muscle cells separated from the endothelium by a continuous basement membrane. The wall thickness was determined as the mean of measurements of two opposite walls. Measurements were taken at the site where the luminal diameter was minimal, because, at this point, measurements are least affected by sectioning angle.

Cross-sections of the aorta descendens in the vicinity of the aortic arc were prepared. Semithin (1 μm) sections were cut, stained with methylene blue and basic fuchsin, and studied planimetrically as mentioned earlier here at a magnification ×25. The external and internal contours of the media were outlined, and the media cross-sectional area, media thickness, and lumen diameter were calculated.

Statistics

Data are given as means ± SEM. Multiple intergroup comparisons were made using the Kruskal–Wallis test followed by the Mann–Whitney *U*-test for pair-wise comparisons. The statistical analysis was carried out using the SPSS software (SPSS Inc., Chicago, IL, USA). The null hypothesis was rejected if P was < 0.05.

RESULTS

Characterization of the model

Some pertinent animal data at the end of the experiment are shown in Table 1. A significant increase in serum creatinine was noted in all SNx groups, and this was paralleled by a decrease in creatinine clearance. Hematocrit values were also lower in all SNx groups compared with sham-operated controls. Systolic BP was significantly elevated in untreated SNx animals as compared with the controls. The increase in BP was prevented by the ACE inhibitor trandolapril. By contrast, no decrease in BP was seen with either the ET_A-selective or -nonselective ET receptor antagonist. The left ventricle weight:body weight ratio was substantially higher in the untreated SNx compared with the sham-operated or trandolapril-treated groups. The groups treated with ET receptor antagonists did not significantly differ from controls. Significantly lower plasma renin activity was found in all SNx groups except the one treated with trandolapril.

Morphological data

The volume density of the myocardial interstitium ($cm^3/cm^3 \times 10^2$) was significantly higher ($P < 0.01$) in the untreated SNx group (2.89 ± 0.16) compared with sham-operated controls (2.15 ± 0.17; Fig. 1 A, B). Trandolapril as well as the selective ET_A receptor antagonist BMS182874 and the nonselective ET receptor antagonist Ro46-2005 prevented ($P < 0.005$) enlargement of the

Table 1. Animal data

	Systolic BP *mm Hg*	LV wt: body wt $\times 10^{-3}$	Serum creatinine *μmol/liter*	Creatinine clearance *ml/min/100 g body wt*	Hct *%*	Plasma renin activity *ng Ang I/ml/hr*
Sham operated $N = 8$	131 ± 5.3[a]	1.98 ± 0.05[a]	30.7 ± 2.5[a]	1.08 ± 0.2[a]	47.3 ± 0.54[a]	13.3 ± 2.92[a]
SNx untreated N = 8	170 ± 8.6	2.23 ± 0.12	64.8 ± 8.5	0.41 ± 0.06	42.2 ± 0.78	1.46 ± 0.22
SNx + trandolapril $N = 8$	128 ± 5.3[a]	1.92 ± 0.08[a]	60.1 ± 3.2	0.43 ± 0.05	39.4 ± 0.74	8.33 ± 2.13[a]
SNz + BMS182874 $N = 8$	154 ± 4.9	2.1 ± 0.04	57.6 ± 4.7	0.5 ± 0.06	39.2 ± 2.19	2.02 ± 0.33
SNx + Ro46-2005 $N = 10$	167 ± 7.6	2.25 ± 0.1	57.2 ± 2.1	0.46 ± 0.03	39.2 ± 1.05	1.62 ± 0.55

Values are means ± SEM. Abbreviations are: LV, left ventrical; Ang I, angiotensin I; SNx, subtotal nephrectomy.
[a] $P < 0.05$ vs. untreated SNx group

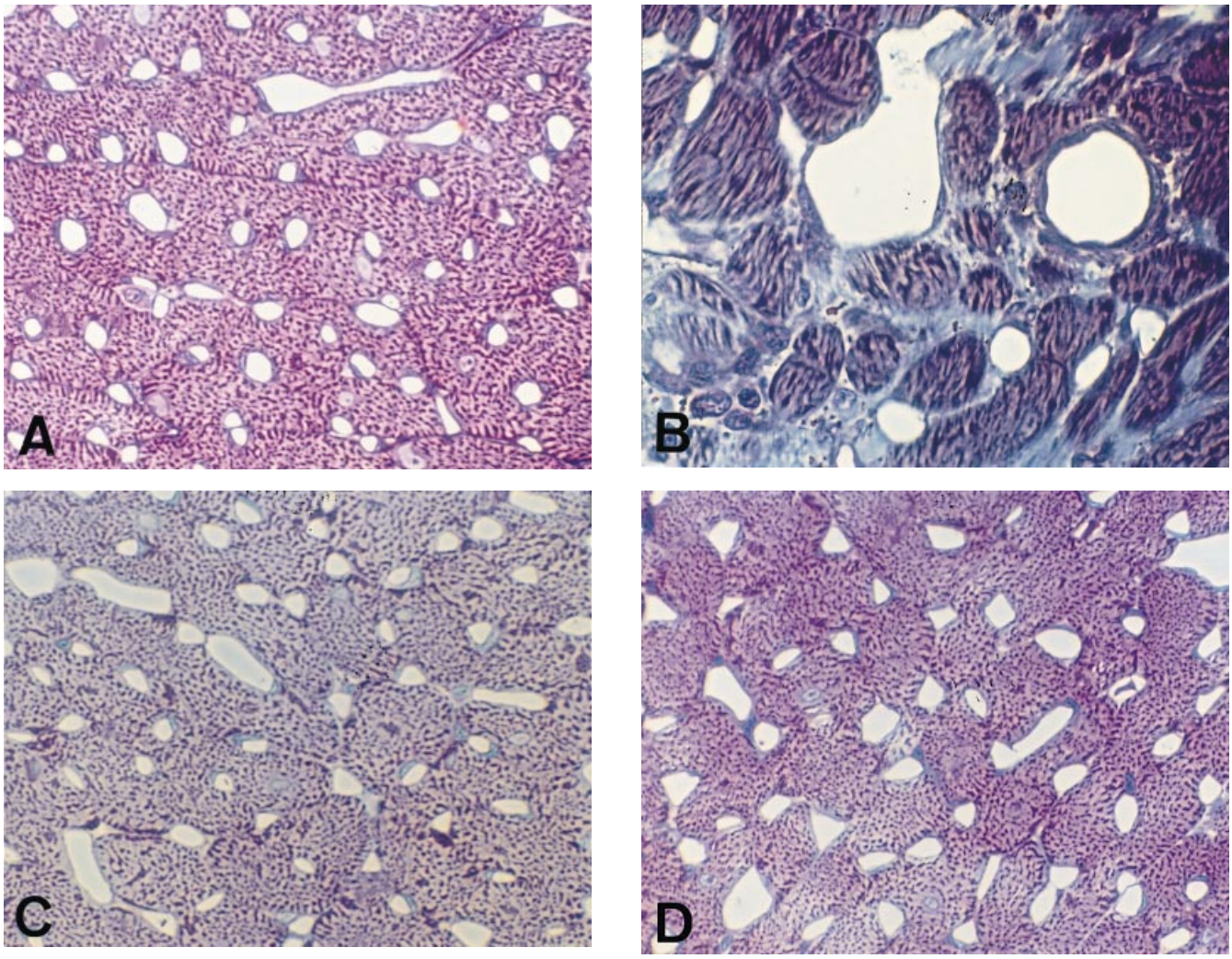

Fig. 1. Structural changes of the myocardium in subtotally nephrectomized (SNx) rats eight weeks after the start of the experiment. Representative findings in sham-operated (*A*), untreated SNx (*B*), BMS182874-treated SNx (*C*), and Ro46-2005-treated SNx animals (*D*). These figures illustrate the increase of interstitial tissue in the untreated SNx animal with the most prominent interstitial expansion (B) and the absence of intramyocardial interstitial enlargement in sham (A) and both groups receiving the ET receptor antagonists (C and D). Semithin section, methylene blue—basic fuchsin (magnification A, C, and D ×640; B, ×1100).

Table 2. Morphometric measurements of small intramyocardial arteries

	Media thickness	Lumen diameter	Wall:lumen ratio
	μm		$\times 10^{m-3}$
Sham operated $N = 8$	1.22 ± 0.04^a	$32{,}6 \pm 0.83$	40 ± 2^a
SNx untreated $N = 8$	1.55 ± 0.07	33.2 ± 0.81	48 ± 3
SNx + trandolapril $N = 8$	1.19 ± 0.04^a	31.9 ± 1.02	40 ± 2^a
SNx + BMS182874 $N = 8$	1.23 ± 0.03^a	35.2 ± 0.69	37 ± 1^a
SNx + Ro46-2005 $N = 10$	1.43 ± 0.07	36.5 ± 1.53^a	40 ± 2^a

Values are means ± SEM.
[a] $P < 0.05$ vs. untreated Snx group

interstitium (2.0 ± 0.16, 2.04 ± 0.15, and 1.77 ± 0.16, respectively; Fig. 1 C, D).

Table 2 summarizes the results of the morphometric analysis of intramyocardial arterioles. A significant thickening of the vessel wall was found in untreated SNx compared with sham-operated controls (Fig. 2 A, B). No increase in the wall thickness was noted in trandolapril- or BMS182874-treated groups (Fig. 2 C, D). The difference between SNx + Ro46-2005 and untreated SNx failed to reach statistical significance. Of note, the mean arterial lumen diameter was highest in the SNx + Ro46-2005 group. Nevertheless, the wall:lumen ratio was significantly lower in all treated groups irrespective of the substance applied.

Table 3 represents the data on aortic structure in the different experimental groups. Cross-sectional area and thickness of the media were substantially higher in untreated SNx (Fig. 3B) when compared with sham-operated controls (Fig. 3A). The wall thickening was partly prevented by trandolapril administration (Fig. 3C). In contrast, the two ET receptor antagonists did not affect aortic wall thickness (Fig. 3 D, E). The changes of wall:lumen ratio went in parallel with the changes of the media.

DISCUSSION

The involvement of ET in the progression of renal insufficiency and conversely a renoprotective role of ET receptor antagonists have been recently recognized in studies on the remnant kidney model [12–15]. Whether ET is also involved in the genesis of cardiovascular complications of renal failure has not been documented so far. The results of this study argue for a role of ET in the structural changes seen in the heart, but not in those occurring in the aorta.

Both in humans and in laboratory animals, uremia is characterized by activation of cardiac fibroblasts followed by intercardiomyocytic interstitial expansion [6, 7, 31]. This increase in interstitial tissue is characterized by a significant increase in mean cell and nuclear volume of interstitial fibroblasts and activation of these cells with increased proliferating cell nuclear antigen positivity, enlargement of the Golgi apparatus, and higher intracellular actin filament content [33]. The expansion of the interstitial tissue could be prevented with ACE inhibitors but not with calcium antagonists or sympathicolytic agents [11]. In this study, we showed significantly lower volume density of the myocardial interstitium after treatment with either ET_A-selective or ET_A/ET_B-nonselective receptor antagonists in moderately severe chronic renal failure. Although the dose–response relationship was not investigated, the effect of the chosen dose of the ET receptor antagonists was comparable with that of a typical dose of an ACE inhibitor.

Considerable information is available on the interaction between ET and cardiac fibroblasts. ETs increase the synthesis of type I and II collagens in adult rat cardiac fibroblasts [34]. In addition, specific binding of ET-1 and ET-3 to cardiac fibroblasts has been documented suggesting the presence of both ET_A and ET_B receptor subtypes [35]. In mouse fibroblasts and in VSMCs of rabbit aorta, long-lasting activation of cation current by very low concentrations of ET-1 was found [36].

In addition, another hallmark of uremia, that is, wall thickening of intramyocardial arteries [3–5], was similarly ameliorated by the ET_A-selective receptor antagonist as by the ACE inhibitor trandolapril. A similar trend was also observed with the nonselective ET receptor antagonist, although the difference was not statistically significant. Wall thickening of intramyocardial arteries in SNx was found to be due to hypertrophy of VSMCs with a significant increase in cell and nuclear volume compared with sham-operated controls [Törnig et al, unpublished data]. Perivascular fibrosis was not involved in the increase of arteriolar wall thickness in renal failure. Apparently, the behavior of blood vessels is quite heterogeneous in renal failure. In another study, we examined the aorta (as a model of an elastic artery) in renal failure and found hyperplasia with only modest hypertrophy of VSMCs [38].

Enhanced expression of ET in the myocardium and vascular endothelium has previously been demonstrated in experimental models of arterial hypertension [21, 39–41] or cardiovascular pathology [29, 42]. Using quantitative polymerase chain reaction, an increased prepro-ET-1 mRNA level has also been recently found in the myocardium of SNx rats (unpublished data). The vasoconstrictive [22] and promitogenic [43, 44] actions of ET might be involved in the genesis of myocardial fibrosis. It is of note that the attenuation of fibrosis was seen, although the two ET receptor antagonists failed to prevent left ventricular hypertrophy in uremic rats. Because both ET_A-selective and ET_A/ET_B-nonselective receptor

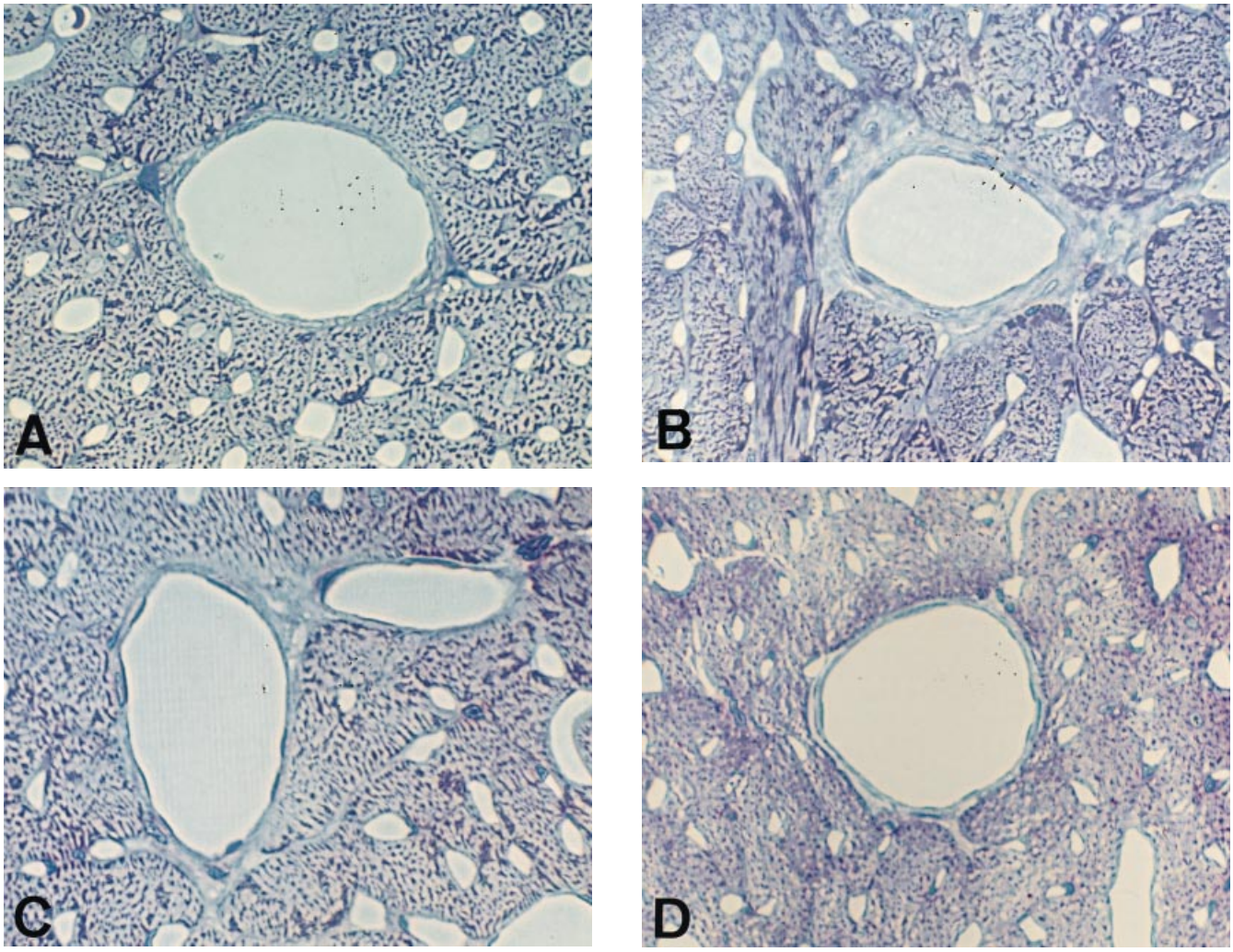

Fig. 2. Structural changes of small intramyocardial arteries in SNx rats eight weeks after the start of the experiment. Representative arteriolar sections of sham-operated (*A*), untreated SNx (*B*), trandolapril-treated SNx (*C*), and BMS182874-treated SNx animals (*D*). The arteriolar wall thickness is considerably greater in SNx (B) than in sham operated animals (A) and animals of the two treated SNx groups (C and D). Semithin section, methylene blue–basic fuchsin (magnification ×640).

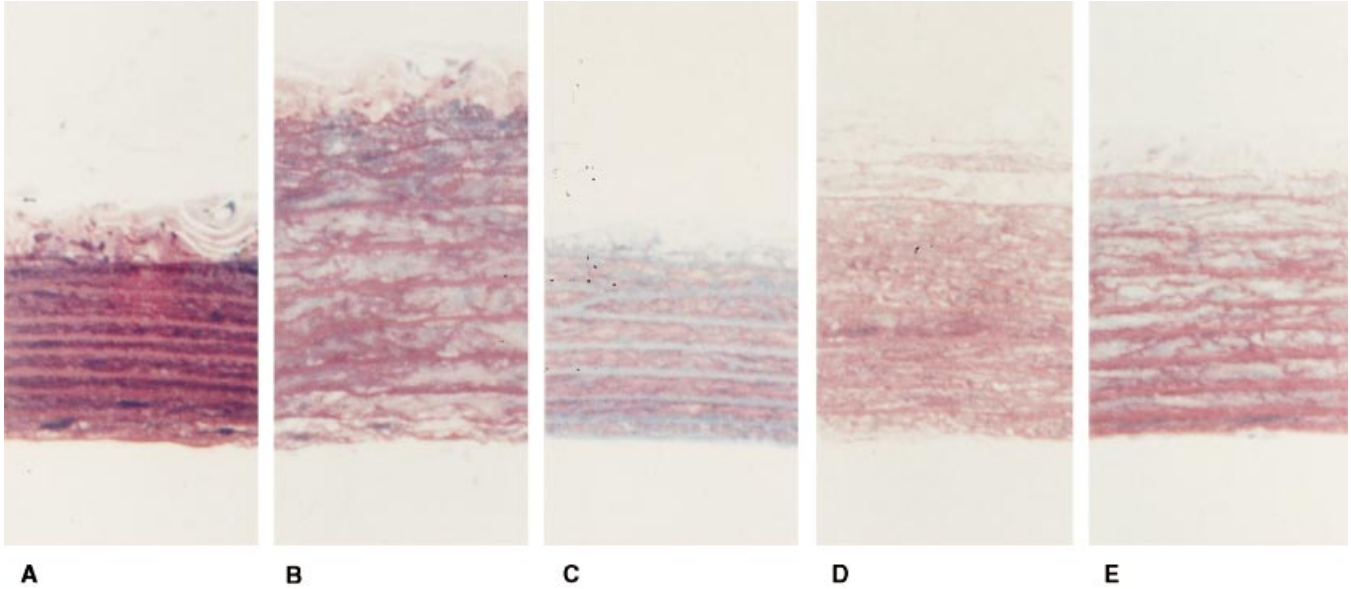

Fig. 3. Structural changes of the aorta in SNx rats eight weeks after the start of the experiment. Representative sections of the aortic wall of sham-operated (*A*), untreated SNx (*B*), trandolapril-treated SNx (*C*), BMS182874-treated SNx (*D*), and Ro46-2005–treated SNx animals (*E*). An increase in media thickness is observed in untreated SNx animals (B). Such an increase is attenuated in SNx animals treated with trandolapril (C), but not in SNx animals treated with either ET receptor antagonist (D and E). Semithin sections, methylene blue–basic fuchsin (magnification ×320).

Table 3. Morphometric parameters of the aorta

	Media cross-sectional area mm^2	Media thickness μm	Lumen diameter mm	Wall:lumen ratio $\times 10^{-3}$
Sham operated $N = 8$	0.51 ± 0.02[a]	84 ± 2[a]	1.85 ± 0.05	45 ± 2[a]
SNx untreated $N = 8$	0.66 ± 0.03	107 ± 5	1.86 ± 0.06	58 ± 3
SNx + trandolapril $N = 8$	0.58 ± 0.06[a]	92 ± 7[a]	1.89 ± 0.04	49 ± 3[a]
SNx + BMS182874 $N = 8$	0.7 ± 0.04	106 ± 4	1.99 ± 0.06	53 ± 2
SNx + Ro46-2005 $N = 10$	0.76 ± 0.03	115 ± 4	1.98 ± 0.04	59 ± 3

Values are means ± SEM.
[a] $P < 0.05$ vs. untreated SNx group

antagonists were equally effective, a specific role of the ET_A receptor is suggested.

Arterial hypertension is considered to be a major cause of vascular remodeling in uremia, although vessel wall thickening cannot be completely prevented by BP lowering [3–5]. This study documents prevention of wall thickening of intramyocardial resistance vessels by the ET_A receptor antagonist independent of BP. This observation is in agreement with some recent data on effects of nonselective ET receptor antagonists in other hypertensive models, for example, DOCA-salt hypertensive rats [45] or DOCA-salt spontaneously hypertensive rats [46]. We admit, however, that we performed plethysmographic BP measurements only. In the absence of telemetric BP monitoring, we cannot exclude some change of the 24-hour-averaged BP level [47]. Nevertheless, the severity of left ventricular hypertrophy went in parallel with the systolic BP values measured by plethysmography, lending further credence to the registered differences in BP between the groups.

One further observation argues against the idea that left ventricular hypertrophy is simply the reaction of the left ventricle to increased afterload. Although the small wall thickness of the right ventricle prevents quantitative measurements with the orientator method, qualitative observations showed cardiomyocyte hypertrophy, expansion of the interstitium and arteriolar wall thickening, as well as expression of renin and ET-1 message and ET-1 protein in the right ventricle as well.

It was not the purpose of this study to elucidate the mechanisms underlying the beneficial cardiac effect of the ET receptor antagonists in this model. Both hypertrophy and hyperplasia of VSMCs have been implicated in the arterial wall thickening in renal failure [38]. ET-1 exerts hypertrophic and mitogenic actions in VSMCs via ET_A receptors [48, 49]. It is reasonable to assume that reversal of such effects played a role in the prevention of the wall thickening of small intramyocardial arteries.

Both ET receptor antagonists and ACE inhibitors have beneficial effects on the cardiovascular abnormalities in renal failure. The question arises as to whether ET and Ang II interact in effecting initiation and progression of the structural changes of the heart. In fact, Ang II increases prepro-ET-1 mRNA expression in endothelial cells [49, 50], VSMCs [51], and cardiomyocytes [20]. Furthermore, Ang II up-regulates ET_B receptors in rat cardiomyocytes [52]. On the other hand, in cultured endothelial cells exposed to ET, conversion of Ang I to Ang II is increased [53]. ACE inhibitors (*a*) prevented hemodynamic changes following administration of exogenous ET to experimental animals [54, 55] and humans [56], (*b*) abolished ET-induced early growth response in isolated myocardial cells [57], (*c*) decreased ET-1 liberation from cultured human endothelial cells [58], and (*d*) reduced ET-1 plasma concentration in hypertensive patients [59]. The Ang II receptor antagonist losartan reduced ET-1 expression in the aorta and peripheral arteries of SNx rats [60]. Conversely, the ET_A receptor antagonist BQ-123 inhibited Ang II–induced contraction of the rabbit aorta [61]. These findings are in line with our observations that ACE inhibitors and ET receptor antagonists have similar effects on the structural changes of the heart in uremia. Based on the previously mentioned observations that indicate interaction of the renin-angiotensin system and ET system, one would anticipate additional benefit from combining ACE inhibitors and ET receptor blockers. A combination of both drugs, however, did not increase the benefit, at least at the doses used in this experiment (unpublished data).

In contrast, additive effects of administration of the ACE inhibitor cilazapril and the mixed ET receptor antagonist bosentan on the mean BP have been recently shown in rats with chronic heart failure [62], and the same appears to be true for prevention of glomerulosclerosis [63].

A potential role of the bradykinin system in the development of cardiac structural changes is supported by the unpublished finding in one of our experimental studies [64] that the bradykinin receptor antagonist HOE 140 prevents left ventricular hypertrophy after SNx.

In contrast to the changes in the heart, the abnormalities in the aortic wall of uremic animals were not affected by either of the ET receptor antagonists used. This observation is in accord with studies performed in DOCA-salt hypertensive rats [65, 66]. Decreased density of aortic ET-1 receptors has been documented in these animals [67] and may explain the inefficacy of ET receptor antagonists in this model. Whether the same is true in experimental uremia is unknown. At any rate, in experimental uremia, the effect of ET receptor antagonists on small resistance arteries is distinctly different from that on large conduit vessels.

In summary, myocardial fibrosis and abnormalities of the structure of small intramyocardial arteries in moder-

ate renal failure are prevented by either an ET_A-selective or an ET_A/ET_B-nonselective receptor antagonist. The beneficial effect of the ET receptor antagonists appears to be independent of changes in systemic BP and left ventricular weight.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin converting enzyme; Ang, angiotensin; BP, blood pressure; ET, endothelin; SNx, subtotally nephrectomized; VSMCs, vascular smooth muscle cells.

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