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Effects of ACE inhibition and bradykinin antagonism on cardiovascular changes in uremic rats

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Background. Cardiovascular death continues to be a major problem in renal failure. Structural abnormalities of the heart and the vasculature contribute to the increased cardiovascular risk. They are ameliorated by angiotensin-converting enzyme (ACE) inhibitors, but because of the nonspecifity of ACE inhibition, it is uncertain whether the beneficial effect is mediated by interfering with angiotensin II (Ang II) or by modulating other effector systems, for example, bradykinin.

Methods. To assess a potential role of bradykinin, subtotally nephrectomized Sprague-Dawley rats (SNX) received either the ACE inhibitor Ramipril (Rami, 0.2 mg/kg body weight p.o.), the specific B2 bradykinin receptor antagonist Hoe140 (0.2 mg/kg body weight, s.c.), or a combination of both, and were compared to sham-operated controls. To separately assess the effect of Ramipril on development and reversal of structural abnormalities, animals were either treated from the third day after SNX or from the fourth week after SNX onward (0.01 mg/kg body weight, p.o.).

Results. Heart and aorta were evaluated by morphometric and stereologic techniques. The weight of the perfused left ventricle, as an index of cardiac hypertrophy, was significantly higher in untreated SNX. While it was significantly lower in animals with early and late Ramipril treatment, the beneficial effect was completely antagonized by Hoe140. The wall-to-lumen ratio of intramyocardial arterioles was significantly higher in untreated SNX compared with controls, but failed to be modified by administration of either Ramipril or Hoe140. In the heart, the intercapillary distance was significantly higher in SNX, but it was not lowered by either early or late Ramipril or Hoe140 treatment. Treatment of SNX with Hoe140 alone, however, resulted in a marked further increase in intercapillary distance. The wall thickness of the aorta was significantly higher in SNX than in controls; early and late Ramipril treatment prevented such increase, and this effect was antagonized by Hoe140.

Conclusion. These findings illustrate that bradykinin plays an important role for the beneficial effect of Ramipril in preventing (and potentially reversing) abnormal cardiovascular structure in uremic hypertensive rats.

Key words: hypertension, renin system, heart, aorta, Ramipril, subtotal nephrectomy.

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Increased cardiovascular mortality continues to be an unresolved problem of chronic renal failure. The genesis is multifactorial, but abnormalities in cardiovascular structure play a major role [1]. The administration of angiotensin-converting enzyme inhibitors (ACEi) significantly improves the structural abnormalities in experimental and clinical studies [2, 3]. In particular, in experimental renal failure left ventricular hypertrophy (LVH), interstitial fibrosis and arteriolar wall thickening were prevented by ACEi, whereas reduced myocardial capillary supply was not affected [4]. Furthermore, in a controlled study, the administration of ACEi was shown to reverse LVH in dialyzed patients [3].

In several experimental models of hypertension, it was shown that ACEi could interfere with vascular remodeling. This action was, at least in part, independent of their hypotensive action [5], since even subhypotensive doses of ACEi were effective [6]. The effect of ACEi was also greater than that seen with equihypotensive doses of alternative antihypertensive agents [7].

Although ACEi were introduced primarily with the intention to reduce the generation of angiotensin II (Ang II), the interpretation of these results remained equivocal because of the restricted specificity of ACEi. In particular, it was uncertain to what extent the effect was explained by accumulation of bradykinin secondary to inhibition of the kininase II activity of the ACE. Bradykinin influences proliferation and protein synthesis in various tissues [8], either per se or secondary to activation of mediators such as nitric oxide (NO) and prostacyclins [9, 10].

The availability of a highly specific B2 antagonist Icatibant or Hoe140 [11] provides a tool to investigate a

potential role of accumulation of bradykinin in the beneficial cardiovascular effects of ACEi. To this end, we compared the effects of the ACEi Ramipril, the specific B2 receptor antagonist Hoe140, and their combination on the following indices: LVH (as reflected by absolute and relative left ventricular weight), myocardial capillary density (as reflected by length density and intercapillary distance), geometry of intramyocardial arterioles, and wall thickness of the aorta.

METHODS

Animals

Forty-eight male Sprague-Dawley rats (weighing 200 g; Invanovas Co., Kisslegg, Germany) were housed in single cages at a constant temperature (20°C) and humidity (25%). The animals were given a high-protein, low-salt diet containing 40% protein and 0.6% NaCl (Altromin C 1002/C 1036; Altromin Co., Lage, Germany). After three days of adaptation, the animals were randomly allocated to partial nephrectomy (SNX) or sham operation (sham). Blood pressure (BP) was measured at the beginning of the experiment and then every two weeks by tail plethysmography.

Renal ablation

First, the right kidney was removed under Ketamin/Diazepam anesthesia (100 mg/kg or 2.5 μg/kg, respectively). Seven days later, the left kidney was partially resected by removing a quantity corresponding to two thirds of the weight of the resected right kidney from the left cortex. The control animals were sham operated by decapsulating the kidney. Special care was taken to avoid damage to the adrenals.

Experimental protocol

The animals were randomly allocated to the following experimental groups (N = 6 to 9 animals per group; administration of medication was started three days postoperatively): (*1*) sham-operated controls (controls), (2) untreated subtotally nephrectomized rats (SNX), (*3*) subtotally nephrectomized rats + 0.1 mg/kg body weight Ramipril (SNX + Rami1), (*4*) subtotally nephrectomized rats + 0.1 mg/kg body weight Ramipril p.o. + 0.2 mg/kg body weight Hoe140 subcutaneously (SNX + Rami1 + Hoe140), and (5) subtotally nephrectomized rats + 0.2 mg/kg body weight Hoe140 subcutaneously (SNX + Hoe140). In an ancillary experiment, medication was started five weeks postoperatively: (6) subtotally nephrectomized rats + 0.2 mg/kg body weight Ramipril p.o. (SNX + Rami2).

Eight weeks after the second operation, the experiment was terminated by perfusion fixation. At the end of the experiment, the abdominal aorta was catheterized under Ketamin/Diazepam anesthesia (doses as mentioned previously in this article). Blood samples were taken, and the viscera were rinsed with 10% dextran solution containing 0.5 g/L Procain-HCl for two minutes. Ten seconds after starting the aortic perfusion, the vena cava was incised to drain the blood. After dextran infusion, the vascular system was perfused with 0.2 mol/L phosphate buffer containing 3% glutaraldehyde for 12 minutes. After the perfusion, the heart of each animal was taken out for determination of weight and volume and tissue sampling, and section staining was performed according to the orientator method [4, 12]. Uniformly random sampling was achieved by preparing a set of equidistant slices of the left ventricle and the interventricular septum with a random start. Two slices were selected by area-weighted sampling and processed using the orientator method [12]. Eight pieces of the left ventricular muscle, including the septum, were prepared and afterward embedded in Epon-Araldite. Semithin sections (1 μm) were stained with methylen-blue and basic fuchsin and were examined by light microscopy with oil immersion and phase contrast at a magnification of 1000:1.

The aorta was cut into a 2 mm thick piece at a certain distance from the aortic arch. After embedding (discussed previously in this article), semithin sections were prepared and stained as described.

Stereological analysis

Quantitative stereology: Heart. All investigations were performed in a blinded manner (that is, the observer was unaware of the study group to which the animal belonged). Per animal, eight random samples of differently oriented left ventricular sections were investigated using the orientator method [4, 12].

In brief, the length density (L_V) of capillaries, that is, the length of capillaries per unit tissue volume, and the volume density (V_V) of cardiac capillaries, defined as the volume of capillaries per unit myocardial tissue volume, were measured in eight systematically subsampled areas per section (57,600 μm^2) using a Zeiss eyepiece with 100 points for point counting. The length density of myocardial capillaries (L_V) was determined using the equation $L_V = 2\ Q_A$, where Q_A is area density (for example, the number of capillary transsects per area of myocardial reference tissue) [13].

Volume density (V_V) of capillaries, interstitial tissue, and myocytes was obtained using the point-counting method [13] according to the equation $P_P = V_V$, where P_P is point density. Reference volume was the total myocardial tissue (exclusive of noncapillary vessels, that is, arterioles and veins). Intercapillary distance, defined as the distance between the centers of two adjacent intramyocardial capillaries, was calculated according to a modification of the formula of Henquell and Honig [4].

Wall thickness and lumen diameter of intramyocardial

Table 1. Chemistry

Groups	S_{Cr}	S_{urea}	Hb	K	Ca	P
	mg/dL		*g/dL*	*mmol/L*		
Controls ($N = 8$)	0.58 ± 0.05	42.4 ± 15.0	15.1 ± 1.28	4.40 ± 0.98	2.48 ± 0.13	2.60 ± 1.13
Untreated SNX ($N = 7$)	0.74 ± 0.05[a]	82.7 ± 20.4[a]	14.8 ± 0.58	4.0 ± 0.23	2.57 ± 0.03[a]	2.41 ± 0.16
SNX + Rami1 ($N = 7$)	0.74 ± 0.07[a]	95.7 ± 39.7[a]	14.8 ± 0.42	4.34 ± 0.43	2.54 ± 0.09	2.28 ± 0.17
SNX + Rami1 + Hoe140 ($N = 7$)	0.72 ± 0.03[a]	83.5 ± 20.3[a]	15.2 ± 0.61	4.15 ± 0.19	2.58 ± 0.06[a]	2.37 ± 0.32
SNX + Hoe140 ($N = 8$)	0.72 ± 0.04[a]	69.9 ± 12.3[a]	16.5 ± 0.84	4.07 ± 0.34	2.57 ± 0.04[a]	2.20 ± 0.37
SNX + Rami2 ($N = 8$)	0.76 ± 0.04[a]	78.6 ± 9.16[a]	16.1 ± 0.82	4.06 ± 0.31	2.59 ± 0.04[a]	2.25 ± 0.35
Analysis of variance	$P < 0.05$	$P < 0.05$	NS	NS	$P < 0.05$	NS

Abbreviations are: S_{Cr}, serum creatinine; S_{urea}, serum urea; Hb, hemoglobulin; SNX, subtotally nephrectomized rats; Rami1, Ramipril. Rami1 is defined as treatment with 0.1 mg/kg body weight Ramipril from day 3 after SNX onward; Rami2 is treatment with 0.2 mg/kg body weight Ramipril from week 4 after SNX onward.
[a] $P < 0.05$ vs. sham-operated control

Table 2. Blood pressure, body weight and weight of left ventricle after perfusion

Groups	Body weight *g*	Systolic blood pressure *mm Hg*	Left ventricular weight *mg*	Left ventricular weight/ body weight ratio *mg/g*
Controls ($N = 8$)	471 ± 21	112 ± 17	943 ± 51	2.01 ± 0.12
Untreated SNX ($N = 8$)	456 ± 40	125 ± 22	1052 ± 109[a]	2.32 ± 0.27[a]
SNX + Rami1 ($N = 7$)	406 ± 43[a,b]	92 ± 15[b]	895 ± 132[b]	2.20 ± 0.15
SNX + Rami1 + Hoe140 ($N = 9$)	414 ± 32[a,b]	104 ± 7[b]	1027 ± 97[a,c]	2.49 ± 0.26[a,c]
SNX + Hoe140 ($N = 9$)	453 ± 18	124 ± 13	1100 ± 93[a,c,d]	2.42 ± 0.14[a,c]
SNX + Rami2 ($N = 7$)	409 ± 31[a,b]	104 ± 14[b]	934 ± 139[b]	2.28 ± 0.23[a]
Analysis of variance	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Rami1 is defined as treatment with 0.1 mg/kg body weight Ramipril from day 3 after SNX onward; Rami2 is treatment with 0.2 mg/kg body weight Ramipril from week 4 after SNX onward. Abbreviations are in Table 1.
[a] $P < 0.05$ vs. sham-operated control
[b] $P < 0.05$ vs. untreated SNX
[c] $P < 0.05$ vs. SNX + Rami1
[d] $P < 0.05$ vs. SNX + Rami2

arteries were determined planimetrically using a semiautomatic image analyzing system (Videoplan, Kontron Co., Eching, Germany). Wall-to-lumen ratio was calculated by dividing wall thickness and lumen diameter [4].

Quantitative stereology: Aorta. Wall thickness, lumen diameter, lumen, and wall area were measured on semithin sections in five to eight animals per group using planimetry, as described in detail [4, 14].

Statistics

All data are expressed as mean ± SD. Kruskal–Wallis test and one-way analysis of variance (ANOVA), respectively, were chosen for analysis of variance, followed by the least-significant difference test to assess the differences between the groups. Results were considered significant if $P < 0.05$.

RESULTS

Animal data

The mean serum creatinine concentrations in SNX animals were only moderately, but significantly elevated compared with sham-operated controls (Tables 1 and 2). This degree of subtotal nephrectomy reduces the nephron number from around 45,000 to 21,700 per kidney [15]. Hemoglobin concentrations were not significantly different between the groups. Systolic BP tended to be higher in untreated SNX than in controls, but this was not statistically significant. BP was significantly lower in both Ramipril-treated SNX groups compared with untreated SNX. This difference was almost completely obliterated by concomitant treatment with Hoe140. In agreement with previous results [4, 15], body weight was significantly lower in both Ramipril-treated SNX groups. The left ventricular weight (after perfusion) was significantly higher in untreated SNX than in controls. This increase was significantly less with early and late Ramipril treatment, respectively. An increased left ventricular weight was seen, however, in animals with Ramipril plus concomitant treatment with Hoe140 or treatment with Hoe140 alone.

Intramyocardial arterioles (morphometrical measurements)

Although the mean luminal diameters were not significantly different, the wall thickness was significantly elevated in SNX animals (Table 3 and Figs. 1 A, B and 2). This was not influenced by treatment with Ramipril (starting either early or late), Hoe140, or their combination (Fig. 1 C, D). The changes of the wall-to-lumen

Table 3. Morphometrical measurements of intramyocardial arterioles

Groups	Wall thickness	Lumen diameter	Wall-to-lumen ratio
	μm		$\mu m/\mu m \times 10^{-3}$
Controls ($N = 9$)	2.56 ± 1.40	41.4 ± 3.93	63 ± 31
Untreated SNX ($N = 8$)	3.49 ± 2.05[a]	42.2 ± 6.74	86 ± 48[a]
SNX + Rami1 ($N = 6$)	3.31 ± 2.58[a]	41.0 ± 2.58	85 ± 64[a]
SNX + Rami1 + Hoe140 ($N = 9$)	3.76 ± 1.74[a]	44.5 ± 5.17	89 ± 47[a]
SNX + Hoe140 ($N = 9$)	3.26 ± 1.86[a]	43.1 ± 4.77	79 ± 45[a]
SNX + Rami2 ($N = 7$)	3.59 ± 1.39[a]	47.8 ± 5.33	86 ± 43[a]
Analysis of variance	$P < 0.05$	NS	$P < 0.05$

NS is not significant.

Rami1 is treatment with 0.1 mg/kg body weight Ramipril from day 3 after SNX onward; Rami2 is treatment with 0.2 mg/kg body weight Ramipril from week 4 after SNX onward.

[a] $P < 0.05$ vs. sham-operated control

ratio went in parallel. As shown in Figure 2, systematic sampling errors resulting from rarefaction of small arterioles or from a systematic failure to measure arterioles of a specific size category are excluded, since the cumulative frequency as a function of luminal diameter remained unchanged (Fig. 2).

Intercapillary distance

Figure 3 shows that the intercapillary distance was significantly higher in untreated and Hoe140-treated SNX compared with sham-operated controls. It was not significantly affected by treatment with Ramipril alone or in combination with Hoe140. Conversely, the length density (mm/mm^3) was significantly less in untreated and Hoe140-treated SNX compared with controls (3.395 ± 558 and 3.303 ± 576 vs. 4.796 ± 913); it was not significantly affected by early and late Ramipril (4.224 ± 887 and 4.318 ± 914) or the combination treatment (4.089 ± 716).

Aorta

Mean wall thickness (Fig. 4) as well as the mean wall thickness/body weight ratio were significantly higher in untreated SNX (125.8 ± 7.4 μm and 0.27 ± 0.01 μm/g) than in controls (101.8 ± 12.9 μm and 0.22 ± 0.02 μm/g, respectively). Aortic wall thickening was due to an increase in aortic smooth muscle cell number (Fig. 5 A, B) and was no longer present with continuous Ramipril treatment (Rami1, 88.6 ± 6.5 μm and 0.22 ± 0.03 μm/g; Fig. 5C). In contrast, in animals additionally treated with Hoe140 (112.1 ± 19.4 μm and 0.27 ± 0.05 μm/g; Fig. 5D) or with Hoe140 alone (111.6 ± 11.4 μm and 0.25 ± 0.02 μm/g), as well as in animals with late Ramipril treatment (Rami2) starting four weeks after SNX (98.6 ± 13.9 μm and 0.25 ± 0.04 μm/g), a beneficial effect could not be observed.

There was no significant difference in aortic lumen and media cross-sectional areas. This was true for absolute values as well as for values factored for body weight (lumen area 4.71 ± 0.46 mm^2 in SNX vs. 4.53 ± 0.76 mm^2 in controls; media area 1.37 ± 0.15 mm^2 in SNX vs. 1.39 ± 0.33 mm^2 in controls). In both of the Ramipril treatment groups, however, the media area was lowest.

DISCUSSION

These results clearly show that in this model of subtotally nephrectomized rat, treatment with the ACE inhibitor Ramipril interfered with the development of the following pathologies: increased left ventricular mass, reduced capillary density, and aortic wall thickening. After ACEi, these indices were lower than in untreated SNX even when Ramipril treatment was delayed, consistent with (but not definite proof for) potential reversal. In contrast, thickening of the wall of intramyocardial arterioles was not affected by ACEi. The beneficial effects of the ACEi on LVH and aortic wall thickening were antagonized by the specific bradykinin B2 receptor antagonist HOE 140. These observations suggest that the beneficial effect of ACEi is mediated, at least in part, via accumulation of bradykinin. The reduction in myocardial capillary supply was further enhanced by treatment with the bradykinin receptor blocker HOE 140, suggesting a potential role of bradykinin on myocardial capillary growth. This finding is in line with previous findings in spontaneously hypertensive rats [16]. In contrast, wall thickening of intramyocardial arterioles remained unaffected by either Ramipril or Hoe140, a finding that argues for the involvement of other mediator systems, for example, endothelin 1 (ET-1) [17].

Several aspects of design and methodology deserve comment. In the present study, resection of renal (cortical) mass, and by implication reduction of nephron number, was deliberately kept modest in order to avoid an important role of confounding factors such as severe hypertension, anemia, and metabolic acidosis. In view of this, it is therefore even more remarkable that we found pronounced abnormalities of cardiovascular structures. For unknown reasons, Ramipril caused a significant reduction in body weight, in agreement with previ-

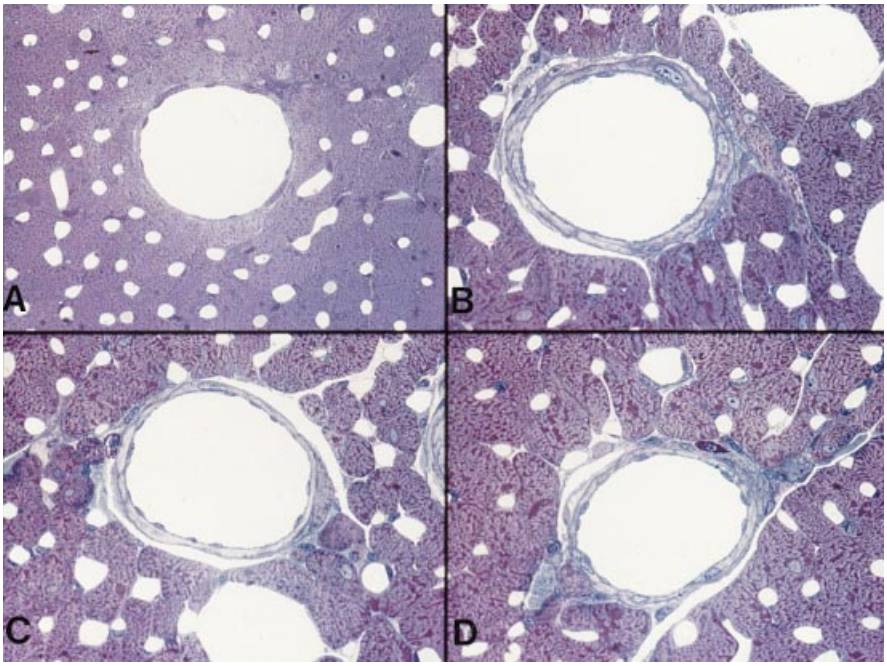

Fig. 1. Changes of intramyocardial arterioles in renal failure: Effect of angiotensin-converting enzyme inhibition (ACEi) and bradykinin antagonism. Semithin sections (magnification, 1:600). The wall thickness is significantly higher in untreated (*B*), ACEi (*C*), and ACEi + Hoe140-treated (*D*) SNX compared with sham-operated controls (*A*).

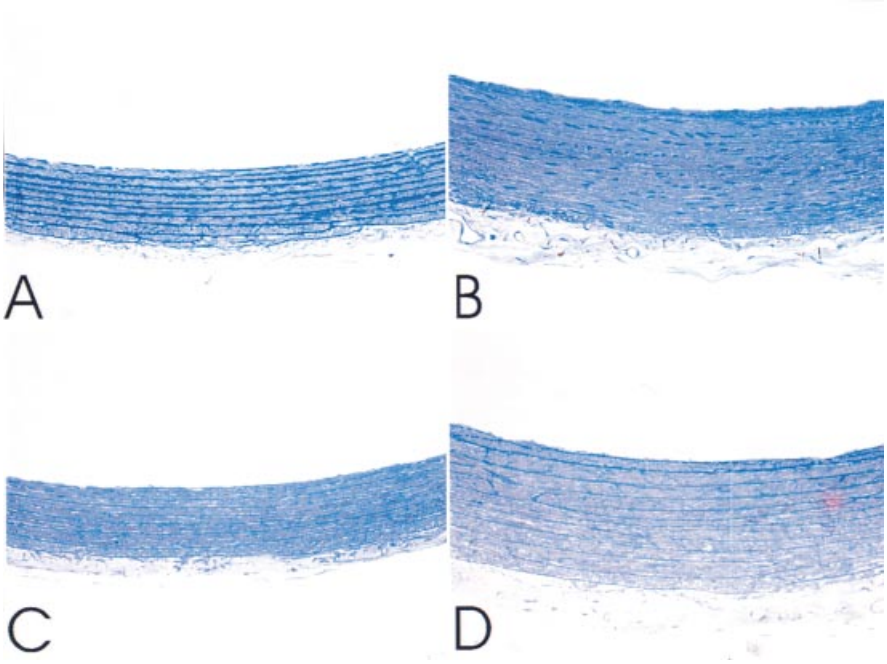

Fig. 5. Effects of Ramipril and Hoe140 treatment on aortic changes in subtotally nephrectomized rats. Semithin sections (magnification, 1:110). Aortic wall thickness is significantly higher in untreated (*B*) and Ramipril + Hoe140 (*D*)-treated SNX compared with controls (*A*) and Ramipril-treated SNX (*C*).

ous studies [4, 15]. To take care of this confounding factor, some of the morphological indices were also factored for body weight.

Blood pressure was measured by tail plethysmography in slightly anesthetized animals. Therefore, when interpreting these results it must be kept in mind that small differences in BP cannot be reliably excluded, as they are demonstrable only by telemetric monitoring [18], particularly in view of the fact that Hoe140 affects the BP-lowering effect of ACEi even in humans [19]. The main finding—obliteration of the beneficial effect of ACEi by B2 receptor blockade—certainly cannot be explained by hypertension as a confounder, since subtotally nephrectomized rats on Ramipril plus Hoe140 had BP values well below those in sham-operated controls.

Both Ramipril and Hoe140 are partially eliminated

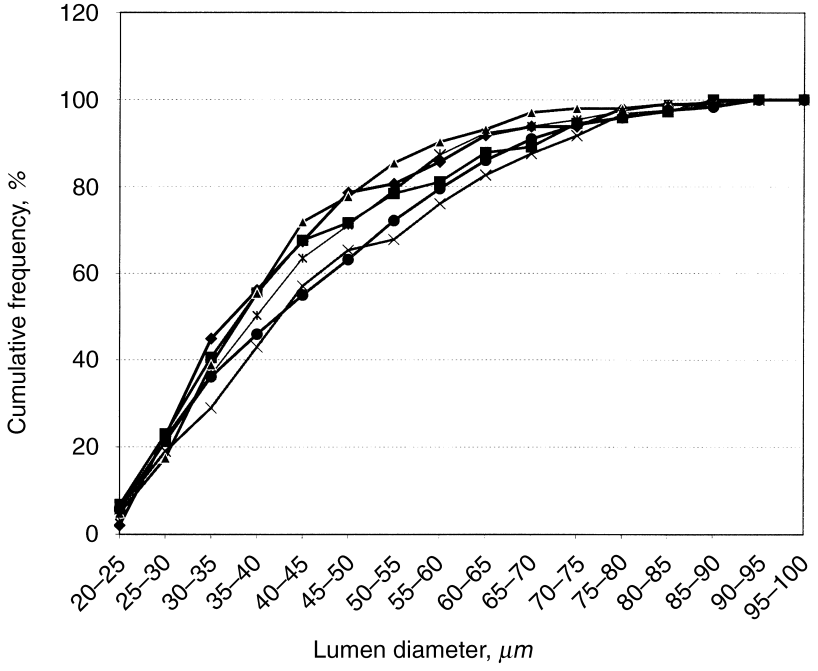

Fig. 2. Cumulative frequency of lumen diameters. There is no significant difference in the cumulative frequency of lumen diameters between the groups. Symbols are: (◆) sham; (■) SNX; (▲) SNX + Ramipril; (×) SNX + Ramipril + Hoe140; (*) SNX + Hoe140; (●) SNX + Hoe140 after 4 weeks.

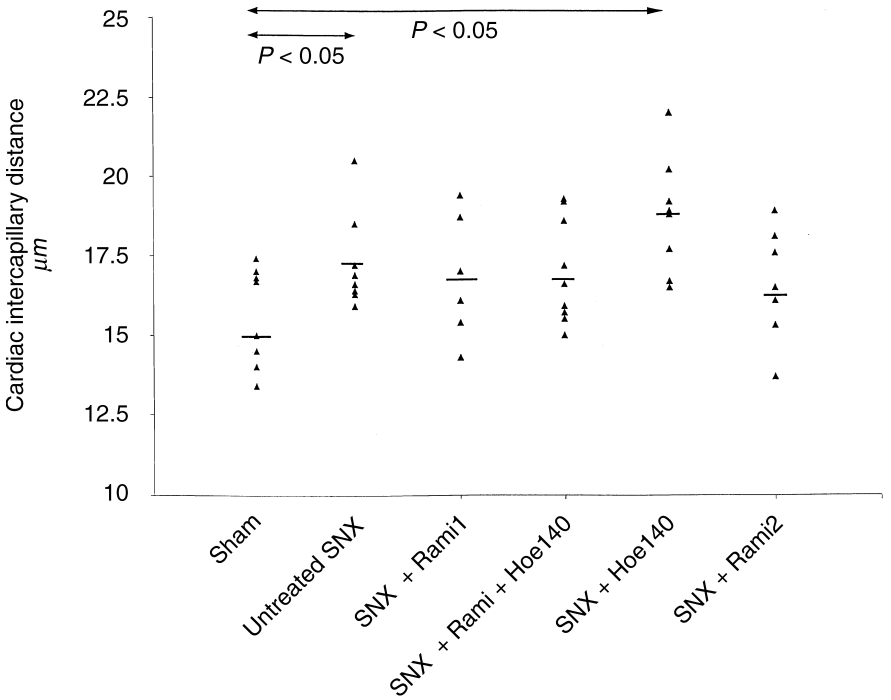

Fig. 3. Individual values and means of cardiac intercapillary distance. Intercapillary distance is significantly higher ($P < 0.05$) in untreated and Hoe140-treated SNX than in sham-operated controls. Treatment with Ramipril alone or in combination with Hoe140 did not significantly modify intercapillary distance.

via renal excretion [11, 20], so that a dose reduction was necessary in the uremic animals. For logistical reasons, only one dose of Ramipril and Hoe140 was used, however.

The protocol, that is, treatment starting at the third day postoperatively, addressed the issue of prevention of structural abnormalities. Our study design thus did not permit definite conclusions concerning regression. Nevertheless, in an exploratory study, we assessed the effects of a delayed Ramipril treatment that started four weeks after SNX. For logistical reasons, a matched control group was not examined.

In this study, a significant increase of left ventricular mass was seen in SNX rats. In previous studies, LVH

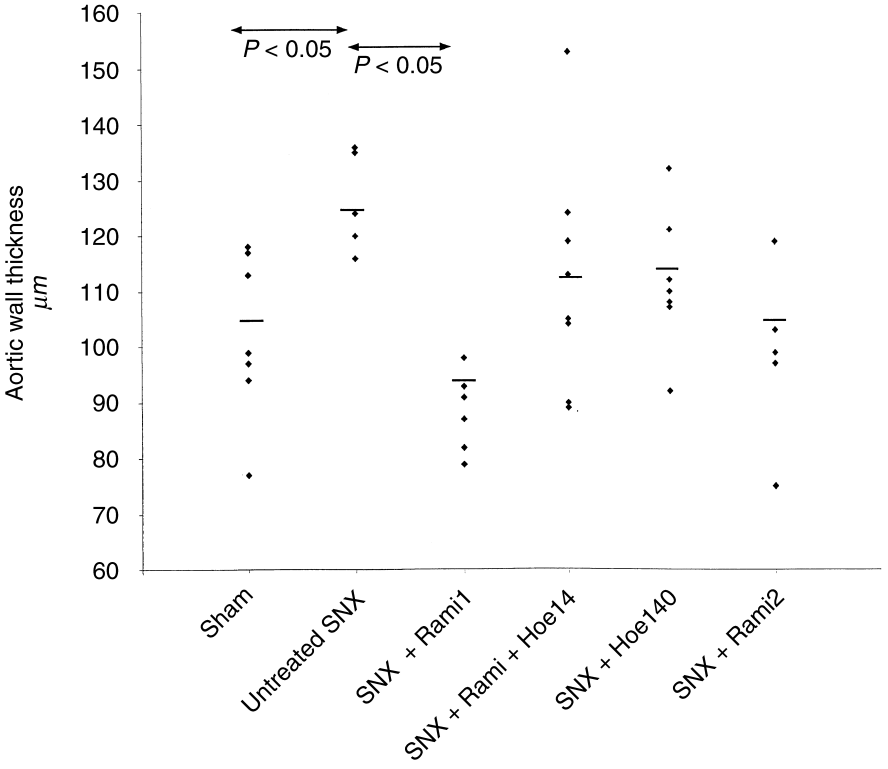

Fig. 4. Individual values and means of aortic wall thickness. Mean aortic wall thickness is significantly higher ($P < 0.05$) in untreated and Ramipril + Hoe140-treated SNX than in sham-operated animals and Ramipril-treated SNX.

could be dissociated from BP, suggesting a role of systemic or local humoral systems [2, 4, 21]. In the present study, Hoe140 prevented the beneficial effect of ACEi on LVH. This observation argues for an important role of bradykinin in the genesis of cardiac hypertrophy. This has also been shown in other animal models, for example, aortic banding [22], although the role of bradykinin on cardiac remodeling has remained controversial [23, 24]. Kohzuki et al failed to find evidence for a role of kinins on the cardioprotective effect of cilazapril in spontaneously hypertensive rats with renal ablation [25]. The discrepancy with the present results may reflect differences in the relative importance of BP elevation for the development of LVH in the two models. A pathogenetic role of bradykinin appears plausible in view of reports on local production of bradykinin [26] and bradykinin accumulation after pretreatment with ACEi in the rat myocardium [27]. Furthermore, B2 receptors had been documented on rat cardiomyocytes [28].

Confirming previous results [4, 29], we found reduced capillary length density and increased intercapillary distance in the hypertrophied myocardium of uremic rats. Ramipril treatment alone caused a numerical increase, which was not statistically significant and which was somewhat lower after concomitant B2 blockade. Treatment with the B2 receptor antagonist Hoe140, however, resulted in a further significant decrease of myocardial capillary supply, pointing to a potential pathogenetic role of bradykinin in cardiac capillarization.

How the hypothetical effect of Ang II on capillary formation is actually mediated is yet unknown. Ang II has opposing actions on microvascular growth [30]: angiotensin II type 1 (AT1) receptors stimulate and AT2 receptors inhibit endothelial cell [31] and vascular smooth muscle cell (VSMC) proliferation. When these cells that normally express only AT1 receptors are transfected with AT2 receptor, the mitogenic action of Ang II in vitro is obliterated [32]. We acknowledge that in the previously mentioned study, the differences of left ventricular mass between the groups are a confounding factor. Indeed, we cannot exclude that changes in capillary density are secondary to differences in left ventricular mass, and more specific studies are required to exclude this possibility.

Wall thickening of arteriolar and arterial vessels is a known feature of uremia [4, 21]. It is independent of BP since wall thickening is seen even if BP is lowered by antihypertensive treatment [21]. There are important differences in the reaction of arterioles and large arteries, that is, intramyocardial arterioles and the aorta, respectively. As expected, the wall-to-lumen ratio of intramyocardial arterioles was significantly higher in SNX animals [4, 20]. Arteriolar wall thickening was also seen in SHR rats [33]. Although in SHR thickening of mesenteric arterioles was prevented by enalapril, in the previously mentioned SNX rats, wall thickness of cardiac arterioles remained unchanged despite treatment with Ramipril.

This is particularly important, since Ramipril had caused the lowering of BP and reduction of LVH. These observations are in agreement with previous studies in our laboratory [4] but differ from those of Kakinuma et al [34], who found lower wall thickness of intramyocardial arteries when rats with chronic renal failure caused by renal artery ligation were treated with ACEi. The absence of an effect of ACEi in our experiment was not modified further by concomitant inhibition of B2 receptors. Taken together, the previously mentioned results argue against a major role of either Ang II or bradykinin but point to a possible role of ET-1, since significantly less thickening of intramyocardial arterioles was noted when SNX rats were treated either with a selective ET_A or a nonselective $ET_{A/B}$ receptor antagonist [17]. The heterogenity of vascular remodeling in different vascular provinces is illustrated by the markedly different findings in the aorta. In the SNX rat, aortic wall thickening could be prevented by treatment with Ramipril. The beneficial effect of Ramipril was attenuated by co-administration of Hoe140. Increased aortic wall thickness is also seen in models of hypertension, for example, SHR and Goldblatt hypertension. In these models, it results from hypertrophy and polyploidy of VSMCs. In contrast, in SNX [21], it involves hyperplasia of aortic VSMCs as well [14]. The beneficial effect of ACEi on aortic wall thickness is in line with previous findings in nonuremic models [5]. The present study extends these findings by documenting a decisive role of bradykinin. As to the potential mechanisms involved, it is of interest that both endothelial cells and VSMCs express bradykinin receptors [35, 36]. Stimulation of endothelial B2 receptors causes release of NO, prostacyclins, and endothelium-derived hyperpolarizing factor (EDHF) [36, 37], which results in vasodilation. Dixon and Dennis postulated a transformation of bradykinin to Des-Arg-bradykinin and stimulation of B1 receptors in VSMCs, which are devoid of B2 receptors, at least under basal conditions [38, 39]. Hoe140 is a specific B2 receptor antagonist, and since B2 receptors are presumably less prevalent in the media, it is possible that the postulated antiproliferative effect of bradykinin was mediated via B2 receptors on endothelial cells. In response to B2 stimulation, they release NO and prostacyclin, known to interfere with proliferation of VSMCs either directly or by interacting with release of platelet-derived growth factor and transforming growth factor-β (TGF-β) from thrombocytes and monocytes, respectively [40]. One further hypothetical mechanism could be the interaction between B2 stimulation and ET-1 secretion. In endothelial cell cultures, diminished secretion of ET-1 in the presence of ACE is observed; this is mediated by a B2-dependent mechanism [41].

Substantial differences between the effect of ACEi in different vascular territories of SNX rats has also been noted by Kakinuma et al [34]. The differences between intramyocardial arteries and the aorta may be due to differences in mediator mechanisms, as hypothesized previously in this article. It is also of interest, however, that wall thickening of intramyocardial arterioles is the result of VSMC hypertrophy [42, 43], whereas thickening of the aortic media is primarily caused by cell hyperplasia [14]. Since the pathogenesis is different, it seems reasonable to anticipate different susceptibilities to interventions.

These results suggest that some of the beneficial effects of ACEi on cardiovascular structures in experimental renal failure are mediated via accumulation of bradykinin. Unless there are major species differences, this finding implies that Ang II receptor antagonists will not duplicate the beneficial effect of ACEi on cardiovascular structure in uremia. Confirmation or refutation of this hypothesis will require direct head-on comparison of these two classes of agents.

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