Abstract We investigated the mechanism of action of the ACE inhibitor–induced increase in cardiac capillary length density. Stroke-prone spontaneously hypertensive rats were treated prenatally and up to the age of 20 weeks with the ACE inhibitor ramipril (0.01 and 1 mg/kg per day PO) and the AT1 receptor agonist losartan (30 mg/kg per day PO). The contribution of endogenous bradykinin potentiation to the ACE inhibitor actions was assessed by cotreatment with the bradykinin B2-receptor antagonist lanatibant (0.5 mg/kg per day, SC via osmotic minipumps) from 6 to 20 weeks of age. At the end of the treatment period, cardiac capillary length density was measured stereologically using the orientator method. The development of hypertension and left ventricular hypertrophy was prevented by high- but not low-dose ramipril and was not affected by chronic bradykinin B2-receptor blockade. Low- and high-dose ramipril significantly increased cardiac capillary length density (3577 ± 279, n = 11 and 3988 ± 300 mm/mm³, n = 10, P < 0.05) compared with vehicle-treated animals (2935 ± 137 mm/mm³, n = 13). These effects were abolished by chronic bradykinin B2-receptor blockade. The bradykinin antagonist alone was without effect on cardiac capillary length density. Losartan prevented hypertension and left ventricular hypertrophy but did not significantly alter cardiac capillary length density (3429 ± 309 mm/mm³, n = 7). Our results demonstrate that chronic ACE inhibitor treatment can increase cardiac capillary length density in stroke-prone spontaneously hypertensive rats independently of a reduction in blood pressure or left ventricular hypertrophy. This effect is related to the ACE inhibitor–induced potentiation of endogenous bradykinin since it was prevented by chronic bradykinin B2-receptor blockade and was not observed following antihypertensive treatment with the AT1-receptor antagonist losartan (Hypertension. 1997;29[part 2]:478–482.)

Key Words • angiotensin-converting enzyme inhibitor • bradykinin • bradykinin antagonist • heart • SHRSP • ramipril • angiotensin • capillary density

The development of hypertension-induced cardiac hypertrophy is characterized by a preferential growth of cardiac myocytes when compared with capillaries.1 Therefore, in hypertrophied hearts, the capillary supply is diminished. Capillary length density in nonhypertrophied hearts from normotensive rats of different ages has been determined recently.2 The authors demonstrated that cardiac capillarization was highest in 5-week-old rats and decreased gradually with age as well as with increasing heart weight. It has also been shown that capillary length density in nonhypertrophied hearts from normotensive animals was higher than in hypertrophied hearts from age-comparable SHR.2,3 On the basis of these and other findings, prevention of LVH by antihypertensive treatment can be expected to normalize capillary density.

ACE inhibitors have gained great clinical importance in the treatment of hypertension and congestive heart failure, and accumulating evidence from clinical trials indicates that these drugs are also beneficial in patients after myocardial infarction.4 Moreover, antihypertensive treatment with ACE inhibitors has been shown to prevent or reduce LVH in hypertensive patients as well as in animal models of hypertension, such as the SHR. In a recent study in SHR, we have demonstrated that early-onset chronic treatment with the ACE inhibitor ramipril improved capillary supply to the heart by increasing cardiac capillary length density even at doses too low to prevent the development of hypertension and LVH.1

The mechanism of action of ACE inhibitors can be explained by either inhibition of ANG II generation, potentiation of endogenous bradykinin due to inhibition of bradykinin degradation, or both.6 Experimental studies have revealed that several organ-protective actions of ACE inhibitors can be related to the bradykinin potentiating action of the drugs.7

In the present study, we aimed to investigate the contribution of bradykinin and ANG II to the ACE inhibitor–induced increase in cardiac capillary length density observed previously in SHR.1 We used the SHRSP model since these animals develop a more pronounced and severe hypertension and LVH and can thus be expected to exert a more pronounced diminution of the capillary supply to the heart than do SHR.

To study the possible mechanisms underlying the ACE inhibitor actions, we compared the effect of the ACE inhibitor ramipril with the AT1-receptor antagonist losartan, which exerts a more specific inhibition of the RAS at the AT1-receptor level, although a possible stimulatory action

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of ANG II on non-AT1 receptors has to be taken into account. In a second approach, we studied the role of bradykinin potentiation on the actions of the ACE inhibitor by a combination treatment with ramipril and the potent and long-acting bradykinin B2-receptor antagonist icatibant.

**Methods**

**Experiment 1**

Male SHRSP, bred at the Department of Pharmacology in Heidelberg since 1975, were used. The animals were treated in utero and subsequently up to 20 weeks of age with the ACE inhibitor ramipril at doses of 1 mg/kg per day (n=10) and 0.01 mg/kg per day (n=11) and with the AT1-receptor antagonist losartan at a dose of 30 mg/kg per day (n=7). Control animals received vehicle (distilled water) (n=13). The drugs were added to the overnight drinking water and carefully adjusted to the individual drinking habits of the growing animals. Dosage of ramipril during pregnancy and lactation was based on body weight of the dams under the assumption of sufficient distribution of the drug into different compartments, including placenta and milk.

Blood pressure was measured by tail plethysmography under light ether anesthesia at 2-week intervals. Measurements were begun when the animals were 6 weeks old.

At the end of the treatment period, the animals were anesthetized with 400 mg/kg chloral hydrate, and the hearts were fixed by retrograde vascular perfusion with 3% glutaraldehyde in 0.2 mol/L phosphate buffer at a pressure of 110 mm Hg. Before fixation, the hearts were flushed with a dextran solution containing 0.5 g/L procaine-HCl. After perfusion, the wet weights of the left ventricles were determined.

The length density of myocardial capillaries (LV(cap/tiss)) was determined using the orientator method. Briefly, the orientator describes an approach to generate isotropic, uniform, random sections of biological specimens that allows the quantitative study of three-dimensional anisotropic structures on two-dimensional sections. The left ventricle of each animal was partitioned systematically into a random set of parallel horizontal slices of equal thickness, parallel to the valvular plane of the heart. Specimens were embedded in Epon/Araldite and 1 μm slices were prepared with an ultramicrotome. The slices were dried at 70°C and stained with methylene blue and alkaline fuchsin. The strong contrast between the optically empty vascular spaces and the surrounding cells allowed the measurement of the sample means of the number of profiles per unit reference area Qx. The length density (Lv) was calculated according to Lx=2Qx. Eight randomized areas per slice, and thus 64 areas per heart, were evaluated.

We preferred the objective three-dimensional parameter of capillarization (capillarity length density) because the conventional parameter capillary density is mathematically not only a function of myocardial capillary supply but is also affected by the orientation distribution of capillary axes with respect to the myofibril axes. From this point of view, the capillary length density excludes effects of the capillary orientations in space on the quantitative capillary data. In hearts of equal size, there is a linear relation between capillary growth and capillary length density. However, it cannot be derived from these parameters, whether capillary growth was realized by neof ormation of additional capillary branches or by pure capillary elongation leading to a more tortuous capillary network.

**Experiment 2: Effect of Chronic Bradykinin B2-Receptor Blockade on the Actions of the ACE Inhibitor**

SHRSP were treated in utero and subsequently up to 20 weeks of age with the following drug combinations: (1) ramipril (1 mg/kg per day) plus icatibant (0.5 mg/kg per day) (n=7), (2) ramipril (0.01 mg/kg per day) plus icatibant (0.5 mg/kg per day) (n=10), (3) vehicle (distilled water) plus vehicle (NaCl 0.15 mol/L) (n=9), (4) vehicle (distilled water) plus icatibant (0.5 mg/kg per day) (n=11).

The bradykinin B2-receptor antagonist icatibant [D-Arg1,(Hyp1, Th1,D-Tic2, Oeo4)-bradykinin] or vehicle (NaCl 0.15 mol/L) was applied chronically by subcutaneous infusion via osmotic minipumps beginning at the age of 6 weeks. Osmotic minipumps were changed every 2 weeks. In a recent study, we demonstrated that losartan at a dose of 0.5 mg/kg per day given by the same route effectively blocked the depressor response to exogenously applied bradykinin. The ACE inhibitors were added to the daily drinking water as described above. Blood pressure was measured by tail plethysmography under light ether anesthesia at 2-week intervals. Measurements were begun when the animals were 6 weeks old.

At the end of the treatment period, cardiac capillary length density as well as left ventricular weight were determined as described in experiment 1.

The study was performed in accordance with the guidelines for animal experiments of the University of Heidelberg and was approved by the German governmental office dealing with animal protection.

**Drugs**

Ramipril and icatibant (Hoe 140) were obtained from Hoechst AG. Losartan was kindly provided by Ronald Smith, DuPont Merck, Wilmington, Del.

**Statistics**

Data are reported as mean±SEM. Statistical analysis was performed by two-way ANOVA followed by appropriate post hoc tests (SYSTAT) between groups. A significance level of P<0.05 was accepted.

**Results**

**Experiment 1: Effect of Chronic ACE Inhibition and AT1-Receptor Antagonism on Cardiac Capillary Length Density in SHRSP**

Oral treatment of SHRSP in utero and subsequently up to 20 weeks of age with ramipril at the low dose of 0.01 mg/kg per day did not affect the development of hypertension and LVH compared with the vehicle-treated control group (0.393±0.019 and 0.391±0.02 g/100 g body weight, respectively). Treatment of SHRSP with both the high dose of 1 mg/kg per day ramipril as well as with losartan at a dose of 30 mg/kg per day delayed and attenuated the development of hypertension and of LVH to a similar extent (0.318±0.004 and 0.286±0.048 g/100 g body weight, respectively) (Fig 1).

Cardiac capillary length density was significantly increased in ramipril-treated rats compared with vehicle-treated animals (Fig 2). This effect was most prominent after high-dose treatment (3988±300 versus 2935±137 mm/mm3 in vehicle-treated controls) but was also observed after low-dose treatment (3577±279 mm/mm3), that is, in SHRSP that had developed full hypertension and LVH. On the other hand, losartan treatment failed to significantly increase cardiac capillary length density (3429±309 mm/mm3) despite the preventive effect of the drug on the development of hypertension and LVH (Fig 2).
Experiment 2: Effect of Chronic Bradykinin B2-Receptor Blockade on the Actions of the ACE Inhibitor

Oral treatment with the high dose of 1 mg/kg per day of ramipril in combination with the subcutaneously applied bradykinin B2 antagonist Icatibant delayed and attenuated the development of hypertension and of LVH (0.281±0.004 versus 0.368±0.005 g/100 g body weight in vehicle-treated controls) (Fig 3). The degree of blood pressure reduction after treatment with high-dose ramipril plus Icatibant was similar to that in experiment 1 in which SHRSP were treated with ramipril alone.

Chronic treatment of SHRSP with the low dose of 0.01 mg/kg per day of ramipril in combination with Icatibant as well as chronic subcutaneous infusion of Icatibant alone did not alter the development of hypertension and LVH compared with vehicle-treated control rats (0.368±0.006, 0.371±0.009, and 0.368±0.005 g/100 g body weight, respectively) (Fig 3).

In combination with the B2-receptor antagonist Icatibant, ramipril failed to increase cardiac capillary length density at the low dose as well as at the high dose despite the effective prevention of hypertension and LVH (3082±284 and 3215±225 mm/mm² for low- and high-dose ramipril-treated rats versus 2923±204 mm/mm² in vehicle-treated controls) (Fig 4). Icatibant by itself had no effect on capillary length density (3042±161 mm/mm²).

Discussion

In the present study, we demonstrate that early-onset long-term treatment of SHRSP with the ACE inhibitor ramipril increases cardiac capillary length density even at subantihypertensive doses. These results confirm our previous observations in SHR using a similar protocol as in the present study. In addition, our findings that the increase in cardiac capillary length density was prevented by chronic bradykinin B2-receptor blockade with Icatibant and that chronic AT1-receptor blockade with Losartan...
failed to significantly increase capillary length density suggesting an involvement of the ACE inhibitor–induced bradykinin potentiation in the capillary growth process.

About 90% of the capillary network is formed postnatally. In the rat, growth of capillaries exceeds growth of the myocardium during the first 11 days of life and stops after 45 days of age, so that capillary density usually decreases with age as well as with increasing heart weight. In the rat, growth of capillaries exceeds growth of the myocardium during the first 11 days of life and stops after 45 days of age, so that capillary density usually decreases with age as well as with increasing heart weight.

Cardiac capillary length density is highest in 5-week-old animals (5189 ± 81 mm/mm³) and decreased gradually with age of the animals (3759 ± 142 and 3525 ± 50 mm/mm³ in 13- and 52-week-old rats, respectively). Therefore, the cardiac capillary length density of 2935 ± 137 mm/mm³ in our vehicle-treated 20-week-old SHRSP reveals a diminished capillary supply compared with age-comparable normotensive rats. Comparison with the data in normotensive rats further shows that in the present study, cardiac capillary length density of SHRSP was shifted to levels of age-comparable normotensive rats. Moreover, the inhibitory effects of losartan on capillary growth could not be compensated for by the effects of the ACE inhibitor.

Several reports have shown that antihypertensive treatment with different ACE inhibitors increased capillary density compared with untreated control animals. Our results demonstrate that the antihypertensive dose of ramipril markedly increased cardiac capillary length density not in the presence of chronic bradykinin B1-receptor blockade despite the effective prevention of LVH. Similarly, losartan failed to significantly increase cardiac capillary length density compared with vehicle-treated SHRSP, although blood pressure and LVH were effectively reduced. The obvious difference in the effect of ramipril and losartan on cardiac capillary length density at doses that produce similar antihypertensive and antihypertrophic actions needs to be explained. First, the ACE inhibitor effects could be due to the bradykinin potentiating action in addition to inhibition of the RAS, while losartan may act more specifically on the RAS by blocking the AT1 receptor. However, there is also some evidence from studies in isolated rat hearts as well as in cell cultures for an interaction between ANG II and the bradykinin/nitric oxide system possibly mediated by an AT2-receptor stimulation. In recent studies, we demonstrated that losartan and ramipril, under identical treatment conditions as in the present study, show strikingly similar effects with regard to an improvement in myocardial function and myocardial metabolic parameters in ex vivo isolated hearts from SHRSP. All these effects of the ACE inhibitor were completely abolished by chronic bradykinin B1-receptor blockade. Thus, the bradykinin-dependent effects of the ACE inhibitor were mimicked by losartan. Moreover, the effects of losartan on coronary flow, an important factor involved in the stimulation of capillary growth, were even more pronounced compared with ramipril, and the drug produced a 2.6-fold higher increase in aortic cGMP content, which can be regarded as a measure of vascular nitric oxide release. It should be noted that all these studies were performed in parallel, so that the results can be easily compared to each other. Therefore, in the present study, losartan could be expected to be at least as effective as ramipril in increasing cardiac capillary length density due to its antihypertrophic effect and to a possible interaction with the bradykinin/nitric oxide system. Therefore, the failure of losartan to increase cardiac capillary length density might involve an additional activation of growth-inhibiting mechanisms and may be related to a chronic overstimulation of non-AT1 receptors such as the AT2 receptor.

Indeed, stimulation with ANG II via AT2 receptors mediates antigrowth effects on coronary endothelial cells. Furthermore, there is evidence that ANG II exerts a mitogenic effect on coronary endothelial cells by an AT1-receptor mechanism. In VSMCs that only express the AT1 receptor, ANG II exerts a growth-promoting action. However, in the presence of AT2 receptors, that is in VSMCs transfected with the AT2 receptor, an antigrowth effect of ANG II could be observed.

In a study in rat cremaster muscle, Munzenmaier and Greene demonstrated that in the microcirculation, the AT1 receptor mediates angiogenic actions while AT2-receptor stimulation causes inhibition of angiogenesis. Therefore, in the present study, losartan treatment might have caused an inhibition of capillary growth by both the inhibition of the AT1 receptor–mediated growth-promoting action of ANG II and by stimulation of the AT2 receptor–mediated antiproliferative action of ANG II. Obviously, the inhibitory effects of losartan on capillary growth could not be compensated for by the effects of the drug on LVH and on cardiac function and metabolism.

On the other hand, the angiogenic action of ramipril was completely abolished by bradykinin B1-receptor blockade, suggesting that endogenous bradykinin is at least a prerequisite of this effect and that ANG II does not contribute to the capillary growth process under these conditions.

However, it is also possible that due to the inhibition of ANG II generation, the ACE inhibitor caused an inhibition of the AT1 receptor–dependent growth-promoting actions of ANG II as well as inhibition of the AT2 receptor–dependent antiproliferative effects of ANG II. Therefore, growth and antigrowth mechanisms may have compensated each other under these conditions, leaving as a net effect the bradykinin-mediated angiogenic action of the ACE inhibitor.
Bradykinin can be involved in the angiogenic process either directly or indirectly, e.g., by increasing coronary flow or by alteration of myocardial metabolism. A direct angiogenic action of bradykinin has been suggested in a study in cultured endothelial cells isolated from coronary venules. In that study, bradykinin (10^{-11} to 10^{-7} mol/L) dose-dependently increased DNA synthesis as evaluated by [3H] thymidine incorporation.26 Bradykinin can also exert an indirect stimulus for capillary growth by a long-term increase in coronary flow and thus by increasing the shear stress--induced release of growth factors involved in the angiogenic process.27 In a recent study, we demonstrated that low-dose ramipril treatment caused an increase in coronary flow and an increase in cardiac contractility independently of its antihypertensive and antihypertrophic action.26 In addition, ACE inhibitor treatment caused a marked alteration in cardiac metabolism as demonstrated by decreased tissue levels of lactate and increased levels of glycerol and the energy-rich phosphates ATP and creatine phosphate.26 In both cases, the effects of the ACE inhibitor were sensitive to bradykinin B_{1}-receptor blockade by icatibant.

A number of stimuli for angiogenesis act by vasodilation, increase in coronary flow, or stretch of the vessel walls. All these potential mechanisms for triggering capillary growth can also be exerted by increased local bradykinin concentration. Thus, activation of these mechanisms by chronic ACE inhibitor--induced bradykinin potentiation may explain the increase in cardiac capillary length density observed in the present study.

One of the most interesting findings of this study is the increased cardiac capillary length density in hearts from low-dose ramipril--treated SHRSP. These animals may benefit from treatment in that their capillary supply and thus oxygen and nutrient delivery as well as metabolite clearance are increased despite the fact that the hearts had developed hypertrophy to a similar extend as hearts from vehicle--treated controls. Again, these effects were completely abolished by bradykinin B_{1}-receptor blockade, suggesting that bradykinin is a prerequisite for this effect. Recent results from a survival study by Linz et al.28 suggest that low--dose ramipril treatment (0.01 mg/kg per day) can increase survival of SHRSP to about 3 months compared with vehicle--treated rats. These results also demonstrate the therapeutic importance of blood pressure reduction and prevention of LVH since high-dose--treated SHRSP lived about 1 year longer than low-dose--treated rats despite a similar effect of both doses on capillary density or on cardiac function and metabolism.

In conclusion, our results demonstrate that early-onset long-term ACE inhibitor treatment increases cardiac capillary length density in SHRSP by a bradykinin--dependent mechanism. In contrast, long-term AT_{1}-receptor blockade failed to alter cardiac capillary length density, presumably by stimulation of AT2 receptor-mediated angiogenesis actions on coronary endothelial cells.

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Blockade of Bradykinin B\textsubscript{2} Receptors Prevents the Increase in Capillary Density Induced by Chronic Angiotensin-Converting Enzyme Inhibitor Treatment in Stroke-Prone Spontaneously Hypertensive Rats

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