Long-term Low-Dose Angiotensin Converting Enzyme Inhibitor Treatment Increases Vascular Cyclic Guanosine 3',5'-Monophosphate

Peter Gohlke, Vera Lamberty, Ingo Kuwer, Susanne Bartenbach, Angela Schnell, Wolfgang Linz, Bernward A. Scholkens, Gabriele Wiemer, Thomas Unger

We investigated functional changes in aortic preparations of spontaneously hypertensive rats treated in utero and subsequently up to 20 weeks of age with the angiotensin converting enzyme (ACE) inhibitors ramipril (0.01 and 1 mg/kg per day) and perindopril (0.01 mg/kg per day). Early-onset treatment with the high dose of ramipril inhibited aortic ACE activity, prevented the development of hypertension, increased aortic vasodilator responses to acetylcholine (10^-8 to 10^-6 mol/L), decreased vasoconstrictor responses to norepinephrine (10^-8 mol/L), and increased aortic cyclic GMP content by 160%. Low-dose ramipril inhibited aortic ACE activity and attenuated the aortic vasoconstrictor response to norepinephrine but had no effect on blood pressure. Low-dose treatment with ramipril and perindopril resulted in a significant increase in aortic cyclic GMP content by 98% and 77%, respectively. Long-term coadministration of the bradykinin B2-receptor antagonist Hoe 140 abolished the ACE inhibitor–induced increase in aortic cyclic GMP. Our data demonstrate that long-term treatment with ACE inhibitors can alter vascular function of compliance vessels independently of the antihypertensive action. The increase in aortic cyclic GMP was due to bradykinin potentiating the action of the ACE inhibitors. (Hypertension. 1993;22:682-687.)

KEY WORDS • angiotensin converting enzyme inhibitors • guanosine cyclic monophosphate • perindopril • ramipril • rats, inbred SHR • bradykinin

Sponstaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) develop hypertension during their first 12 weeks of life. The increase in blood pressure is associated with functional changes of the vascular wall. It has been reported that the endothelium-dependent relaxation of blood vessels to different agonists is impaired in hypertensive compared with normotensive animals.1-6 Endothelium-derived relaxing factor (EDRF) can be released by the endothelium in response to a number of agonists, including acetylcholine, bradykinin, and norepinephrine (for review, see References 7 and 8). EDRF causes vascular relaxation by stimulation of soluble guanylate cyclase, leading to a rise in the intracellular cyclic GMP (cGMP) content. The formation of cGMP in rabbit aortic segments and bovine endothelial cells can be stimulated in a concentration-dependent fashion by the angiotensin converting enzyme (ACE) inhibitor ramipril.9 This effect may be the result of an ACE inhibitor–induced potentiation of endogenous kinins, leading to enhanced EDRF release and, subsequently, cGMP formation.

Previous studies in SHR have shown that early-onset ACE inhibitor treatment prevented the development of hypertension and vascular hypertrophy in several vascular beds10-14 and also improved the endothelium-dependent responses in the aorta of SHR.6,12 Whether these effects are invariably associated with the antihypertensive action of ACE inhibitors or can be dissociated from blood pressure in that they can be observed also with subantihypertensive doses of ACE inhibitors has not yet been determined. Therefore, in the present study we investigated the effect of long-term oral high-dose (antihypertensive) and low-dose (subantihypertensive) treatment with ACE inhibitors on aortic function in SHR.

Methods

Experiment 1

SHR (n=12 per group) were obtained from Mollegard, Skensved, Denmark, and 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 (group 1) and 0.01 mg/kg per day (group 2). Control animals received vehicle (water) (group 3). 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.15 Blood pressure was measured at 2-week intervals by tail plethysmography with rats under light ether anes

Received March 11, 1993; accepted in revised form June 28, 1993.

From the Department of Pharmacology and German Institute for High Blood Pressure Research, University of Heidelberg (P.G., V.L., I.K., S.B., A.S., T.U.), and Hoechst AG (W.L., B.A.S., G.W.), Frankfurt, Germany.

Correspondence to Peter Gohlke, PhD, Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, 69120 Heidelberg, FRG.
Effect of Early-Onset Treatment With Ramipril on Systolic Blood Pressure in Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>126±3</td>
<td>135±5</td>
<td>148±3</td>
<td>163±4</td>
<td>171±3</td>
<td>176±6</td>
<td>182±5</td>
<td>181±5</td>
</tr>
<tr>
<td>Ramipril, 0.01 (mg/kg)/d</td>
<td>119±4</td>
<td>125±4</td>
<td>141±4</td>
<td>155±3</td>
<td>173±4</td>
<td>182±4</td>
<td>178±5</td>
<td>180±3</td>
</tr>
<tr>
<td>Ramipril, 1 (mg/kg)/d</td>
<td>122±4</td>
<td>122±4</td>
<td>120±4</td>
<td>120±4</td>
<td>117±5</td>
<td>118±4</td>
<td>120±4</td>
<td>116±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Systolic blood pressure was measured by the tail-cuff method and is in millimeters of mercury.

Enzyme Activity

Experiment 2: Aortic Angiotensin Converting Enzyme Inhibitors and Vascular Wall Thesia. Measurements were begun when the animals were 6 weeks old.

Functional study. At the end of the treatment period, the descending thoracic aorta was excised and dissected free of connective tissue. The aorta was divided into 2-mm wide rings and cut off in small strips. The strips were suspended in a 10-mL organ bath containing Krebs-bicarbonate buffer (mmol/L: NaCl, 113.8; NaHCO₃, 22; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄, 1.1; CaCl₂, 2.5; and glucose, 5.5). The solution was continuously gassed with 95% O₂-5% CO₂ to adjust pH to 7.4 and was maintained at 37°C. Contractions of the strips were recorded isometrically with a load of 1 g on the tissues.

After an equilibration period, the constricting effect of norepinephrine (10⁻⁸ mol/L), angiotensin (Ang) I (10⁻⁴ to 10⁻¹ mol/L), or Ang II (10⁻⁸ mol/L) added to the organ bath was measured. Aortic relaxation by acetylcholine (10⁻⁸ to 10⁻⁶ mol/L) added to the organ bath was determined in aortic strips precontracted with norepinephrine (10⁻⁸ mol/L).

Experiment 2: Aortic Angiotensin Converting Enzyme Activity

SHR (n=12 per group) were treated in utero and up to 20 weeks of age as described in Experiment 1. At the end of the treatment period, the descending thoracic aorta was excised, dissected free of connective tissue, and rapidly frozen in liquid nitrogen. Aortas were pulverized by mortar and pestle and transferred into 0.3% Triton X-100 and sonified for 10 seconds. After centrifugation (6000 rpm for 20 minutes) ACE activity was measured by a fluorometric method using Z-Phe-His-Leu as substrate.¹⁶

Experiment 3: Cyclic GMP Determination

SHRSP bred at the Department of Pharmacology in Heidelberg were used for the study. The animals were treated in utero and subsequently up to 20 weeks of age with the following drug combinations (n=12 per group): (1) 1 mg/kg per day ramipril plus vehicle (0.9% NaCl), (2) 1 mg/kg per day ramipril plus Hoe 140 (500 μg/kg per day), (3) 0.01 mg/kg per day ramipril plus vehicle (0.9% NaCl), (4) 0.01 mg/kg per day ramipril plus Hoe 140, (5) 0.01 mg/kg per day perindopril plus vehicle (0.9% NaCl), (6) 0.01 mg/kg per day perindopril plus Hoe 140, (7) vehicle (water) plus vehicle (0.9% NaCl), and (8) vehicle (water) plus Hoe 140.

The bradykinin B₂-receptor antagonist Hoe 140 (0-[D-Arg₄,Hyp⁴,Thi⁵,D-Tic⁶,Oic⁷]-bradykinin) or vehicle (0.9% NaCl) was applied chronically to 6-week-old rats by subcutaneous infusion via osmotic minipumps, which were changed every 2 weeks. In a recent study we demonstrated that Hoe 140 at a dose of 500 μg/kg per day effectively blocked the depressor responses to exogenously applied bradykinin.¹⁷ The ACE inhibitors were added to the daily drinking water as described above.

At the end of the treatment period, the thoracic aorta was excised, dissected, and snap-frozen in liquid nitrogen. The tissue was pulverized by a cell disruptor and transferred into 1N formic acid/acetone (15:85 vol/vol). After centrifugation, an aliquot of the supernatant was analyzed for cGMP with a radioimmunoassay kit (Du Pont de Nemours, NEN Division, Dreieich, Germany). Protein determinations were performed according to Lowry et al.¹⁸

Drugs

Ramipril and Hoe 140 were obtained from Hoechst AG, Frankfurt/Main, Germany. Perindopril was obtained from Servier, Courbevoie, France.

Statistics

Data are reported as mean±SEM. Statistical analysis was performed by two-way analysis of variance followed by appropriate post hoc tests (CRISP) between groups. A significance level at a value of p<.05 was accepted.

Results

Experiment 1

Blood pressure. Oral treatment of SHR in utero and subsequently up to 20 weeks of age with the high dose of 1 mg/kg per day ramipril completely prevented the development of hypertension, whereas SHR treated with the low dose of 0.01 mg/kg per day ramipril developed hypertension in parallel to the control group (Table).

Vascular function. Aortic ACE activity was significantly attenuated in both treated groups, as demonstrated by the inhibition of the concentration-dependent aortic contraction in response to Ang I (Fig 1). Aortic contraction to Ang II was not affected (Fig 1).

Aortic contraction to norepinephrine (10⁻⁸ mol/L) was significantly inhibited in the high- and low-dose ramipril-treated groups (Fig 2).

Acetylcholine caused a concentration-dependent relaxation of precontracted aortic strips, which was markedly enhanced in the high-dose ramipril-treated group (Fig 3). In the low-dose ramipril-treated group, this effect was observed only at the low concentration range of acetylcholine (10⁻⁸ mol/L) but not at higher acetylcholine concentrations (Fig 3).

Experiment 2

ACE activity in aortic tissue homogenates was dose-dependently reduced in high- and low-dose treated groups by 68% and 40%, respectively (Fig 4).
Experiment 3

Aortic cGMP content. The effect of early-onset ACE inhibitor treatment alone or after coadministration with Hoe 140 on aortic cGMP content is shown in Fig 5. Values are expressed as percent difference from controls. Aortic cGMP concentration in untreated control animals was 58.4 ± 5.8 fmol/mg protein. Low-dose treatment with ramipril and perindopril resulted in a significant increase in aortic cGMP content by 98% and 77%, respectively (Fig 5). This effect was even more pronounced in the high-dose ramipril-treated group (160% increase).

Coadministration of the bradykinin B2-receptor antagonist Hoe 140 completely abolished the ACE inhibitor-induced increase in aortic cGMP (Fig 5). Given alone, the bradykinin antagonist had no effect.

Discussion

Our results show that long-term early-onset treatment with ACE inhibitors altered vascular function of compliance vessels in SHR as demonstrated by the decreased vasoconstrictor response of aortic strips to norepinephrine, the increased vasodilator response to acetylcholine, and the increased aortic cGMP content.

It is well established that vascular function is altered in hypertension. Impaired dilator responses to several agonists including acetylcholine have been observed in aortic preparations of different animal models of hypertension. The degree of impairment of vascular function in hypertension is determined by the intensity and duration of hypertension. Recent studies have shown that the vasodilator responses to acetylcholine decreased with age in aortas of both SHR and Wistar-Kyoto rats but to a greater extent in the hypertensive strain. In a study by Shimamura et al, the endothelium-dependent relaxation of aortas from three strains of SHR (SHR, SHRSP, and malignant SHRSP), which differ in their degree of hypertension, was investigated. The authors demonstrated an impairment of the endothelium-depen-
ACE inhibitors can prevent vascular hypertrophy in different vascular beds, including rat aorta. 10-11-23 Thereafter studies revealed that antihypertensive treatment with aortic contraction capacity. Furthermore, the reduction response to norepinephrine could be due to a reduced arteries of SHR as demonstrated by a reduction in the ACE inhibitor treatment did not generally attenuate the treatment.

14 In addition, a number of other media-to-lumen ratio as well as the number of smooth muscle cell layers.14 In addition, a number of other studies revealed that antihypertensive treatment with ACE inhibitors can prevent vascular hypertrophy in different vascular beds, including rat aorta. 10-11-23 Therefore, a reduced contractility of the aortic wall in response to norepinephrine could be due to a reduced smooth muscle mass after long-term ACE inhibitor treatment.

On the other hand, because the vasocostrictror response to Ang II was not different between ACE inhibitor–treated and vehicle-treated groups, the ACE inhibitor treatment did not generally attenuate the aortic contraction capacity. Furthermore, the reduction of the aortic vasoconstriction to norepinephrine after low-dose ACE inhibitor treatment as observed here is unlikely to be merely due to structural changes because we have recently shown in a study using the same regimen of drug administration that vascular hypertrophy was not affected by long-term low-dose ACE inhibitor treatment. 14 Therefore, additional mechanisms such as alterations in adrenergic transmission, receptor number or affinity, or the prevention of hypertension-induced damage of the endothelium have to be considered.

First, ACE inhibition is known to cause prejunalional and postjunalional sympathoinhibitory effects (for review, see Reference 24) most likely due to the inhibition of Ang II formation, thereby eliminating the facilitatory effect of Ang II on noradrenergic neurotransmission. In this respect it should be noted that aortic ACE activity was inhibited not only after high-dose but also after low-dose ACE inhibitor treatment. This was demonstrated indirectly by the inhibition of the aortic constrictror response to Ang I as well as by direct measurement of ACE activity in aortic tissue homogenates. Because of the strong and tight binding of ramiprilat to ACE with a dissociation half-life of 640 minutes, the ACE inhibition is still measurable even under these ex vivo conditions. 25

Second, norepinephrine may stimulate EDRF synthesis by an \( \alpha \)-adrenergic receptor–mediated mechanism.7 Constrictor responses to \( \alpha \)-adrenergic agonists in several arteries are potentiated by the removal of the endothelium as well as by pretreatment with inhibitors of soluble guanylate cyclase, eg, methylene blue or the nitric oxide scavenger hemoglobin. 26-27 Thus, norepinephrine can exert an endothelium-mediated vasodilatator response that may counteract its direct vasoconstrictor effect. Hypertension development could lead to an imbalance between both mechanisms due to damage to the endothelium. Furthermore, hypertension-induced structural changes leading to an enlargement of the subendothelial space can increase the diffusion distance of EDRF. 20 In view of the very short half-life of EDRF, 28 the enhanced diffusion time will diminish its vascular effects.

Low-dose ACE inhibitor treatment also increased aortic vasorelaxation induced by acetylcholine, although this effect was only significant at the low concentration of acetylcholine (10^{-5} mol/L). The fact that low-dose ACE inhibitor treatment failed to enhance aortic relaxation in response to higher concentrations of acetylcholine could be interpreted to mean that low-dose ACE inhibitor treatment does not markedly influence aortic vasodilator capacity. However, recent findings demonstrated that acetylcholine at higher concentrations (3x10^{-7} to 10^{-5} mol/L) can also cause endothelium-dependent contraction in the aorta of adult SHR by releasing a cyclooxygenase-dependent endothelium-derived contracting factor.4 Thus, the release of an endothelium-dependent contracting factor at high concentrations of acetylcholine may have counteracted the increase in aortic vasorelaxation induced by low-dose ACE inhibitor treatment.

To investigate the mechanisms underlying the effects of ACE inhibitor treatment on vascular function in more detail, we measured aortic cGMP content of SHRSP after early-onset long-term treatment with
bradykinin B₂-receptor antagonist Hoe 140. Together, these data suggest that endothelial cells are able to release bradykinin. In keeping with this idea is a recent study demonstrating a local formation and release of bradykinin in bovine endothelial cells. The source of vascular cGMP in our study remains to be identified. Studies in human umbilical vein as well as in porcine aortic endothelial cells demonstrated that endothelial cells are capable of producing cGMP. 

Thus, cGMP production by the endothelium may have contributed to the increased cGMP levels in whole aortic homogenates measured in the present study. On the other hand, Rapoport and Murad demonstrated a time- and concentration-dependent increase in cGMP in rat thoracic aorta after exposure to acetylcholine that was completely prevented by removal of the endothelium before but not after addition of acetylcholine. These findings suggest that cGMP production was stimulated in smooth muscle cells of the media with the stimulus derived from the endothelium.

In summary, our results demonstrate that early-onset long-term treatment with ACE inhibitors can alter vascular function of compliance vessels independently of the effects on blood pressure. The increase in aortic cGMP could be prevented by long-term coadministration of a bradykinin B₂-receptor antagonist, indicating that it was due to a bradykinin-potentiating action of the ACE inhibitors.

References


phenypropyl-L-alanyl)-(lS,3S,5S,)-2-azabicyclo(3.3.0)octane-3-carboxylic acid (Hoe498) in rat, dog and man. *Drug Res.* 1984;34:1435-1447.


Long-term low-dose angiotensin converting enzyme inhibitor treatment increases vascular cyclic guanosine 3',5'-monophosphate.

P Gohlke, V Lamberty, I Kuwer, S Bartenbach, A Schnell, W Linz, B A Schölkens, G Wiemer and T Unger

Hypertension. 1993;22:682-687
doi: 10.1161/01.HYP.22.5.682

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/22/5/682