

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

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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 B_2 -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

Spontaneously 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 Møllegaard, 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-

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Effect of Early-Onset Treatment With Ramipril on Systolic Blood Pressure in Spontaneously Hypertensive Rats

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

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

thetia. 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 (10⁻⁶ to 10⁻⁸ mol/L), or Ang II (10⁻⁸ mol/L) added to the organ bath was measured. Aortic relaxation by acetylcholine (10⁻⁶ to 10⁻⁸ 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 (D-Arg,¹[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-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).

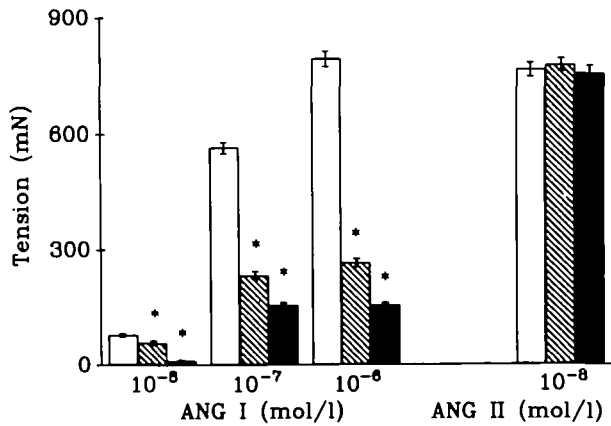


FIG 1. Bar graph shows aortic contraction to angiotensin I (ANG I) and angiotensin II (ANG II) after early-onset treatment of spontaneously hypertensive rats with ramipril at doses of 0.01 mg/kg per day (hatched bars) and 1 mg/kg per day (solid bars). Control animals received vehicle (white bars). **P* < .05.

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

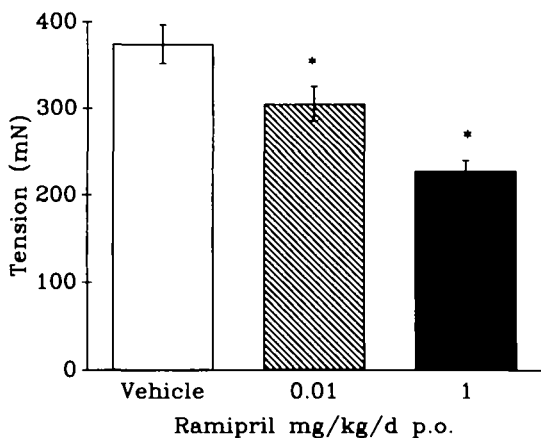


FIG 2. Bar graph shows aortic contraction to norepinephrine (10⁻⁸ mol/L) after early-onset treatment of spontaneously hypertensive rats with ramipril at doses of 0.01 mg/kg per day (hatched bars) and 1 mg/kg per day (solid bars). Control animals received vehicle (white bars). **P* < .05.

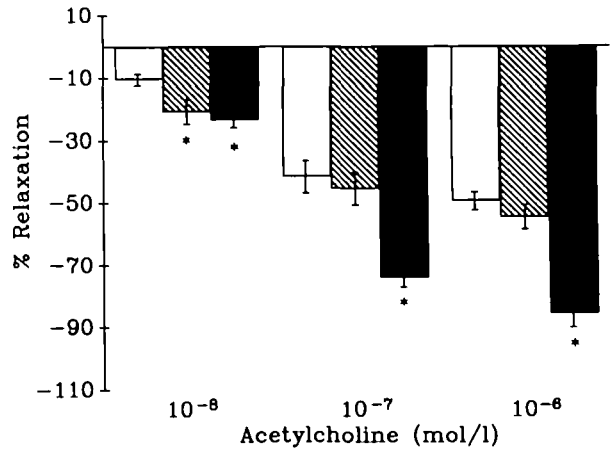


FIG 3. Bar graph shows aortic relaxation to acetylcholine after early-onset treatment of spontaneously hypertensive rats with ramipril at doses of 0.01 mg/kg per day (hatched bars) and 1 mg/kg per day (solid bars). Control animals received vehicle (white bars). **P* < .05.

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.^{2-6,19-22} 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.^{6,22} In a study by Shimamura et al,⁶ 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-

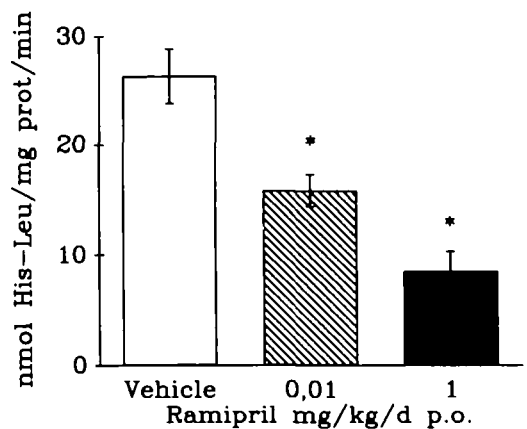


FIG 4. Bar graph shows angiotensin converting enzyme activity in aortic homogenates after early-onset treatment of spontaneously hypertensive rats with ramipril at doses of 0.01 mg/kg per day (hatched bars) and 1 mg/kg per day (solid bars). Control animals received vehicle (white bars). **P* < .05.

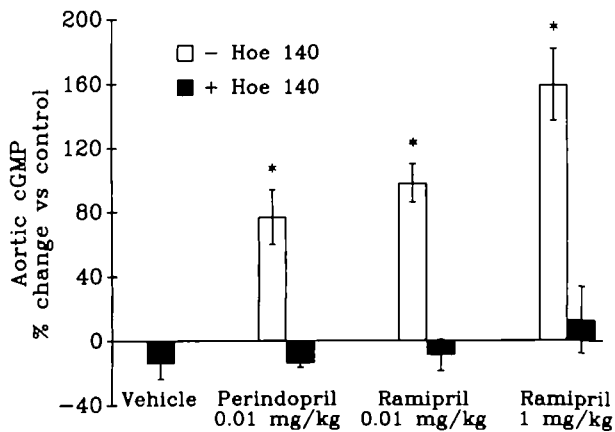


FIG 5. Bar graph shows aortic cyclic GMP (cGMP) content after early-onset treatment of spontaneously hypertensive rats with ramipril (0.01 and 1 mg/kg per day), perindopril (0.01 mg/kg per day), and vehicle. The bradykinin B_2 -receptor antagonist Hoe 140 (500 μ g/kg per day) (solid bars) or vehicle (white bars) were coadministered by subcutaneous infusion starting at the age of 6 weeks. * $P < .05$.

dent relaxation to acetylcholine that was closely related to the level of hypertension. Several studies suggested that normalization of blood pressure can restore the endothelium-dependent responses.^{6,12,19,21}

The mechanisms underlying these changes in agonist-induced vascular responses in hypertensive animals are still controversial. They may include changes in the synthesis, release, or metabolism of EDRF as a result of hypertension-induced changes in the endothelium as well as structural alterations of the vascular wall or the concomitant release of an endothelium-derived contracting factor (for review, see References 1 and 8).

In the present study, the aortic vasoconstriction to norepinephrine was reduced and the aortic relaxation to acetylcholine was increased by high-dose ACE inhibitor treatment. These changes in vascular function may be largely explained by the preventive effects of high-dose ACE inhibitor treatment on hypertension and vascular hypertrophy.

We did not measure the effect of ACE inhibitor treatment on vascular structural alterations. However, in a recent study using a similar study design and drug regimen, high-dose ACE inhibitor treatment prevented the development of vascular hypertrophy in mesenteric arteries of SHR as demonstrated by a reduction in the 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 vasoconstrictor 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 prejunctional and postjunctional 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 constrictor responses to Ang I as well as by direct measurement of ACE activity in aortic tissue homogenates. Because of the strong and tight binding of ramipril 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 α_2 -adrenergic receptor-mediated mechanism.⁷ Constrictor responses to α_2 -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 vasodilator 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^{-8} 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 (3×10^{-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

ramipril (0.01 and 1 mg/kg per day) and perindopril (0.01 mg/kg per day). Our results show that low-dose treatment with both ACE inhibitors increased aortic cGMP content. This effect was more pronounced after high-dose ramipril treatment.

Bioassay experiments suggested that the basal release of EDRF is decreased in aortas with intact endothelium from SHR compared with aortas from Wistar-Kyoto rats.²⁹ In a study by Shirasaki et al,²² it was shown that basal cGMP levels were not different in young SHR and Wistar-Kyoto rats but were decreased in 15- to 18-week-old SHR compared with age-matched Wistar-Kyoto rats together with a reduced endothelium-dependent aortic relaxation induced by acetylcholine. Thus, during the development of hypertension, the function of the endothelium to mediate dilator responses of the vasculature decreases in association with changes in vascular cGMP levels. In experimentally induced hypertension, the attenuated increase in cGMP in response to acetylcholine could be reversed by restoring the blood pressure to normal.³⁰

Therefore, in our study the correction of hypertension by antihypertensive treatment also may have corrected hypertension-induced changes in vascular cGMP content. However, our results clearly demonstrate that low-dose ACE inhibitor treatment led to an increase in aortic cGMP level without affecting blood pressure. Furthermore, the alterations in aortic cGMP by low- and high-dose ACE inhibitor treatment were completely abolished by long-term blockade of bradykinin B₂-receptors. Therefore, it is conceivable that in the present study the increase in vascular cGMP was due to the ACE inhibitor-induced inhibition of bradykinin breakdown.

Bradykinin and acetylcholine are known to stimulate EDRF release^{31,32} and to increase aortic levels of cGMP, an effect that could be prevented by removal of the endothelium (for review, see Reference 7).

In vitro, the addition of the ACE inhibitor perindoprilat to the organ bath of several vessel preparations including rat thoracic aorta did not lead to vascular relaxation under basal conditions, suggesting that the ACE inhibitor did not directly stimulate the release of EDRF from endothelial cells.³³ However, the ACE inhibitor potentiated the endothelium-dependent relaxation in response to bradykinin (10⁻¹¹ to 10⁻⁷ mol/L), probably by decreasing bradykinin breakdown. Similarly, in isolated canine coronary artery rings with intact endothelium, addition of the ACE inhibitor perindoprilat enhanced the relaxation to bradykinin as well as the bradykinin-induced stimulation of cGMP production but did not affect basal cGMP levels.³⁴

On the other hand, a study by Hecker et al³⁵ in bovine coronary arteries demonstrated that the ACE inhibitor moexiprilat not only potentiated the bradykinin-induced cGMP increase but also increased basal cGMP levels when applied alone. Similar results were obtained in human umbilical vein endothelial cells, showing that ACE inhibitor treatment increased resting intracellular Ca²⁺ and cGMP concentrations.^{35,36} These effects could be abolished by preincubation of the cells with the 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.³⁷

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.^{36,38} 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 Murad³⁹ 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.

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