Effects of Cilazapril on Cerebral Vasodilatation in Hypertensive Rats

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Endothelium-dependent dilatation of cerebral arterioles is impaired during chronic hypertension. The goal of this study was to determine the effects of an angiotensin converting enzyme inhibitor, cilazapril, on endothelium-dependent dilatation in pial arterioles. Four-month-old Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP) received cilazapril in their drinking water (500 mg/L) for 3 to 6 months. Treatment with cilazapril reduced mean arterial pressure in both WKY rats and SHRSP and had no significant effect on baseline diameter of pial arterioles measured with a cranial window. Responses to bradykinin and A23187, but not to nitroglycerin and adenosine, were impaired in SHRSP. Cilazapril did not affect responses to bradykinin (3×10⁻⁷ M) and A23187 (10⁻⁶ M) in WKY rats but significantly increased cerebral vasodilatation in response to bradykinin (52±4% vs 27±5%) and A23187 (19±3% vs 8±3%) in SHRSP. Cilazapril also tended to increase dilator responses to nitroglycerin and adenosine in SHRSP. In another group of SHRSP, treatment with cilazapril for 4 days produced a moderate reduction in blood pressure and increased cerebral vasodilatation in response to bradykinin, A23187, and adenosine. Topical application of the active form of cilazapril (cilazaprilat) for 40 minutes also increased cerebral vasodilatation in response to bradykinin, A23187, and nitroglycerin in SHRSP. The data indicate that an angiotensin converting enzyme inhibitor enhances cerebral vasodilatation in response to endothelium-dependent agonists in SHRSP and may also increase responses to endothelium-independent agonists. (Hypertension 1993;22:150-155)

Key Words • angiotensin converting enzyme inhibitor • rats, inbred SHR, stroke-prone • cerebral arteries • endothelium-derived relaxing factor • vasodilation • bradykinin • cilazapril
responses to various agonists were tested. All rats were housed in similar conditions and had free access to water.

At the time of study, animals were anesthetized with pentobarbital sodium (50 mg/kg IP). After a tracheotomy, animals were ventilated mechanically with room air and supplemental oxygen. Gallamine triethiodide (15 to 30 mg/kg IV) was used for skeletal muscle paralysis. Supplemental anesthetic and skeletal muscle relaxant were administered as needed (pentobarbital sodium, 10 to 20 mg·kg\(^{-1}\)·h\(^{-1}\) IV; gallamine triethiodide, 5 to 10 mg·kg\(^{-1}\)·h\(^{-1}\) IV).

A catheter was placed in the left femoral vein for injection of drugs. The femoral arteries were cannulated for measurement of arterial blood pressure and for withdrawal of blood for measurement of arterial blood gases and pH. A cranial window was prepared over the right parietal cortex and suffused with artificial cerebrospinal fluid (CSF) bubbled continuously with a gas mixture. In artificial CSF, pH was 7.33±0.01; PCO\(_2\) was 42±1 mm Hg, and PO\(_2\) was 76±3 mm Hg in WKY rats; in SHRSP, pH was 7.35±0.01, PCO\(_2\) was 43±1 mm Hg, and PO\(_2\) was 73±3 mm Hg (mean±SEM). Temperature was maintained at approximately 38°C. Arterial blood gases and pH were monitored periodically and maintained within normal limits (pH, 7.45±0.01; PO\(_2\), 35±1 mm Hg; PO\(_2\), 107±4 mm Hg in WKY rats; pH, 7.49±0.01; PCO\(_2\), 34±2 mm Hg; PO\(_2\), 111±2 mm Hg in SHRSP).

Pial arteriolar diameter was measured with a video image shearing device (model 907, Instrumentation for Physiology & Medicine, San Diego, Calif).

### Experimental Protocol

Cerebral vessels were superfused with artificial CSF for 30 minutes before application of agonists. In each rat, we studied responses of the largest pial arteriole present in the cranial window. All drugs were dissolved in artificial CSF and then superfused over the cranioectomy. Application of vehicle did not affect vessel diameter. With the exception of A23187, the diameter of cerebral arterioles was measured immediately before application of each agonist and every 20 to 30 seconds for 2 to 4 minutes during application of agonists. Steady-state responses to agonists were reached within 1 to 2 minutes. Values obtained at steady state are reported in this study. Because responses of pial arterioles to A23187 are slower than responses to other agonists, the diameter of cerebral arterioles was measured after continuous superfusion with A23187 for 10 minutes. Several experiments (n=3) indicated that application of agonists did not alter pH of artificial CSF in the cranial window.

To study effects of topical application of the ACE inhibitor, we pretreated pial arterioles with cilazaprilat (10\(^{-6}\) M) for 40 minutes before application of other agonists.

Effects of long-term (3 to 6 months), short-term (4 days), and acute (40 minutes) topical treatment with the ACE inhibitor on responses of pial arterioles to the vasoactive agonists were determined. We examined responses of cerebral arterioles to two endothelium-dependent agonists (bradykinin and A23187) and two endothelium-independent agonists (nitroglycerin and adenosine) in all study groups.

### Results

#### Arterial Pressure and Baseline Vascular Diameter

Effects of cilazapril on mean arterial pressure and baseline diameter are shown in the Table. In untreated WKY rats and SHRSP, mean arterial pressure was 105±4 and 202±5 mm Hg, respectively (mean±SEM). Long-term treatment with cilazapril reduced blood pressure to 78±3 mm Hg in WKY rats and 107±2 mm Hg in SHRSP. Thus, long-term treatment with cilazapril reduced arterial blood pressure in SHRSP to a level similar to that in untreated WKY rats. Treatment with cilazapril for only 4 days produced a moderate reduction of blood pressure in SHRSP (Table). Blood pressure in SHRSP treated with topical cilazapril was not different from that in untreated SHRSP (Table).

Long-term and short-term treatment with cilazapril had no significant effect on baseline diameter of pial arterioles (Table). In addition, topical application of cilazapril did not change baseline diameter.

### Responses to Bradykinin and A23187

Long-term treatment with cilazapril had no effect on responses to bradykinin or A23187 in WKY rats (Figs 1 and 2). In contrast, cerebral vasodilatation in response...
to bradykinin and A23187 was increased in SHRSP after long-term and short-term treatment with cilazapril. Topical application of cilazapril increased responses to bradykinin and A23187 in SHRSP (Figs 1 and 2). Topical cilazapril increased cerebral vasodilation to bradykinin but had no effect on responses to A23187 in WKY rats. Thus, treatment with an ACE inhibitor may improve endothelium-dependent cerebral vasodilation in SHRSP regardless of the duration of treatment. In addition, it also appears that effects of the ACE inhibitor are not specific for bradykinin, because cerebral vasodilation in response to A23187 was also improved in SHRSP after treatment with cilazapril.

Responses to Nitroglycerin and Adenosine

Long-term treatment with cilazapril increased cerebral vasodilation in response to nitroglycerin (10⁻⁶ M) and adenosine (10⁻⁴ M) in SHRSP but not in WKY rats (Figs 3 and 4). Treatment with cilazapril for 4 days also increased vasodilation produced by adenosine (10⁻⁴ M) in SHRSP. During topical application of cilazaprilat, nitroglycerin produced more vasodilation in SHRSP than in untreated SHRSP. Thus, treatment with an ACE inhibitor may enhance endothelium-independent cerebral vasodilation in chronically hypertensive animals.

**Discussion**

There are two major findings in this study. First, treatment with an ACE inhibitor restored cerebral vasodilator responses to the endothelium-dependent agonists bradykinin and A23187 toward normal in SHRSP. Second, responses of pial arterioles to the endothelium-independent agonists nitroglycerin and adenosine also tended to be potentiated after long- and short-term treatment with cilazapril and after topical treatment with cilazaprilat in SHRSP. These findings suggest that effects of the ACE inhibitor on dilator responses in SHRSP are not specific for endothelium-dependent agonists and are not related primarily to decreases in blood pressure or structural changes in the vessel wall.

**Consideration of Methods**

Endothelium-dependent responses of the cerebral microcirculation have been examined in several labora-

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**FIG 1.** Bar graphs show effects of treatment with angiotensin converting enzyme inhibitor on dilatation of pial arterioles in response to bradykinin in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP). *P<.05 vs response under control conditions. Control, untreated animals (WKY, n=15; SHRSP, n=16); Long-Term, treatment with cilazapril for 3 to 6 months (WKY, n=18; SHRSP, n=15); Short-Term, treatment with cilazapril for 4 days (SHRSP, n=7); Topical, topical application of cilazaprilat for 40 minutes (WKY, n=7; SHRSP, n=8).

**FIG 2.** Bar graphs show effects of treatment with angiotensin converting enzyme inhibitor on dilatation of pial arterioles in response to A23187 in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP). *P<.05 vs response in control animals. Control, untreated animals (WKY, n=16; SHRSP, n=16); Long-Term, treatment with cilazapril for 3 to 6 months (WKY, n=16; SHRSP, n=15); Short-Term, treatment with cilazapril for 4 days (SHRSP, n=7); Topical, topical application of cilazaprilat for 40 minutes (WKY, n=6; SHRSP, n=8).
Topically applied agonists appear to penetrate smooth muscle, reach endothelium, and cause release of endothelium-derived vasoactive factors. This conclusion is based on the findings that responses of cerebral arterioles to acetylcholine, bradykinin, and A23187 are inhibited after selective injury to the endothelium⁷⁻⁸ and that inhibitors of synthesis of endothelium-derived relaxing factor (nitric oxide) synthase attenuate dilatation in response to acetylcholine and ADP.¹¹,¹³ Bioassay experiments provide strong evidence for endothelium-dependent release of endothelium-derived relaxing factor in response to topically applied agonists.¹²

**Endothelium-Dependent Vasodilatation**

Relaxation of cerebral blood vessels in response to bradykinin and A23187 is endothelium-dependent.⁷⁻⁸,¹¹,¹³⁻¹⁵ Dilatation of pial arterioles in response to these agonists is impaired in chronically hypertensive rats.¹

Effects of ACE inhibitors on endothelium-dependent responses have been studied in arteries from normotensive and hypertensive animals.⁵⁻⁹,¹⁰ In chronically hypertensive rats, treatment with an ACE inhibitor improves endothelium-dependent relaxation of the aorta in response to acetylcholine in vitro.⁶ It has been proposed that an ACE inhibitor may improve endothelium-dependent vasorelaxation by a mechanism not related to its blood pressure-lowering effects, because treatment with hydralazine, at a dose that produced a similar decrease in arterial pressure, had no effect on responses to acetylcholine.⁶ Studies in normotensive animals also suggest that ACE inhibitors can potentiate relaxation to some endothelium-dependent agents by a mechanism unrelated to changes in blood pressure.⁹,¹⁰

Acute changes in blood pressure can influence responses of cerebral blood vessels to vasoactive stimuli.¹⁶ In this study, long- and short-term treatment with an ACE inhibitor in SHRSP produced different levels of reduction in blood pressure, whereas local application of cilazaprilat had no effect on blood pressure. Similar improvement in cerebral vasodilatation in SHRSP in response to bradykinin and A23187 was observed after both long- and short-term treatment with cilazapril and nitroglycerin (M)

**FIG 3.** Bar graphs show effects of treatment with angiotensin converting enzyme inhibitor on dilatation of pial arterioles in response to nitroglycerin in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP). *P<.05 vs response in control animals. Control, untreated animals (WKY, n=16; SHRSP, n=17); Long-Term, treatment with cilazapril for 3 to 6 months (WKY, n=18; SHRSP, n=15); Short-Term, treatment with cilazapril for 4 days (SHRSP, n=7); Topical, topical application of cilazaprilat for 40 minutes (WKY, n=7; SHRSP, n=8).

**FIG 4.** Bar graphs show effects of treatment with angiotensin converting enzyme inhibitor on dilatation of pial arterioles in response to adenosine in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP). *P<.05 vs response in control animals. Control, untreated animals (WKY, n=17; SHRSP, n=13); Long-Term, treatment with cilazapril for 3 to 6 months (WKY, n=17; SHRSP, n=16); Short-Term, treatment with cilazapril for 4 days (SHRSP, n=7); Topical, topical application of cilazaprilat for 40 minutes (WKY, n=7; SHRSP, n=8).
during topical application of cilazaprilat. Thus, improved cerebral vasodilatation in hypertensive animals after ACE inhibition is not necessarily related to reduction of blood pressure.

The mechanism by which ACE inhibition increases vasodilatation in response to bradykinin and A23187 in SHRSP is not known. Increased dilation of pial arterioles in response to bradykinin after treatment with cilazapril may be due in part to inhibition of the breakdown of bradykinin by ACE. Our findings with A23187, however, indicate that treatment with cilazapril improves endothelium-dependent cerebral vasodilatation by a mechanism that is not specific for responses to bradykinin. In WKY rats, long-term treatment with the ACE inhibitor did not potentiate cerebral vasodilatation in response to bradykinin. Two possible factors may account for the absence of potentiated cerebral dilator responses to bradykinin in WKY rats after long-term treatment with the ACE inhibitor. First, the ACE inhibitor produced a moderate reduction of arterial pressure in WKY rats, which could impair dilator responses of pial arterioles. Normal responses to A23187, nitroglycerin, and adenosine after treatment with the ACE inhibitor make this possibility unlikely. Second, the findings could result from less ACE activity in WKY rats than in SHRSP. Metabolism of bradykinin would not be affected as much in WKY rats as in SHRSP if ACE activity is lower in WKY rats than in SHRSP. Based on previous studies, it is not clear whether ACE activity in cerebral vessels is lower in WKY rats than in SHRSP.

Because a renin-angiotensin system (including ACE) appears to be present in brain, we considered the possibility that ACE inhibition has an influence on nonvascular cells within the cranial window and thus indirectly affects vascular responses. Although we cannot completely exclude such a possibility, the finding that treatment with cilazapril at had no effect on baseline diameter of cerebral arterioles suggests that, if there were changes in neural and glial metabolism, they were not sufficient to produce changes in vascular diameter.

**Endothelium-Independent Vasodilatation**

Treatment with the ACE inhibitor tended to potentiate cerebral vasodilatation in response to nitroglycerin and adenosine in SHRSP but had little effect in WKY rats. Dilation of pial arterioles in response to nitroprusside and adenosine is not changed after selective injury to endothelial cells. Thus, dilation of pial arterioles in response to nitrovasodilators and adenosine is not endothelium dependent.

The effect of ACE inhibitors on endothelium-independent vasodilatation is controversial. Captopril potentiates endothelium-independent relaxation of the aorta from normotensive rats in response to sodium nitroprusside in vitro. Both long- and short-term treatment with cilazapril improve relaxation of the aorta from SHR in response to acetylcholine. In the same study, short-term treatment with cilazapril had no effect on relaxation in response to nitroprusside, and the effect of long-term treatment with cilazapril on responses to nitroprusside was not reported. The absence of increased response to a nitrovasodilator after short-term treatment with cilazapril in SHR is similar to our findings. Perindoprilat, another ACE inhibitor, potentiated endothelium-dependent relaxation produced by bradykinin, acetylcholine, and thrombin in the canine basilar artery. It was not reported whether perindoprilat potentiates endothelium-dependent relaxation in the basilar artery without affecting endothelium-independent relaxation.

In the present study, potentiation of endothelium-independent responses to nitroglycerin and adenosine in SHRSP was not consistent, and it was difficult to discern a pattern of the altered responses. Nevertheless, altered responses to endothelium-independent agonists have been observed in several situations and other studies, and the phenomenon is not likely due to experimental artifacts. Different degrees of ACE inhibition by cilazapril at various treatment intervals might contribute to this inconsistency.

The mechanism by which cilazapril potentiated endothelium-independent cerebral vasodilatation is not clear. Relaxation of vascular smooth muscle involves changes in intracellular calcium concentration. ACE inhibitors appear to affect the influx of calcium in smooth muscle through calcium channels and thereby change vessel tone in hypertensive rats. It is possible that cilazapril potentiated endothelium-independent cerebral vasodilatation by affecting the handling of calcium ions and calcium permeability in smooth muscle cells.

Long-term treatment with ACE inhibitors may produce structural changes in the vessel wall, which may then influence vascular responses. Our findings during topical application of cilazaprilat, however, indicate that structural changes alone do not account for increased responses to vasodilators in SHRSP.

In summary, treatment with an ACE inhibitor improves endothelium-dependent cerebral vasodilatation in SHRSP. Cerebral vasodilatation in response to nitroglycerin and adenosine also appears to be enhanced by the ACE inhibitor in SHRSP. Effects of the ACE inhibitor do not appear to be related primarily to effects on blood pressure. We speculate that effects of the ACE inhibitor may be related in part to an unidentified cellular effect on vascular smooth muscle.

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