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 were impaired in SHRSP. Cilazapril did not affect responses to bradykinin (3 × 10^{-7} M) and A23187 (10^{-7} 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

Endothelium-dependent relaxation is impaired during chronic hypertension, but treatment of hypertension restores endothelium-dependent relaxation in vitro toward normal. Cerebral vasodilatation in response to bradykinin and A23187 (a calcium ionophore) appears to be endothelium dependent. Responses of cerebral arterioles to bradykinin and A23187 are impaired in stroke-prone spontaneously hypertensive rats (SHRSP). The first goal of this study was to examine effects of long-term antihypertensive treatment with cilazapril, an angiotensin converting enzyme (ACE) inhibitor, on endothelium-dependent cerebral vasodilatation in SHRSP. We used A23187 to determine whether effects of the ACE inhibitor were specific for bradykinin, and thus might be related to effects on bradykinin metabolism, or whether responses to another agonist also might be altered. Effects of the ACE inhibitor on responses to endothelium-independent agonists also were examined.

In normotensive animals, ACE inhibitors may potentiate vascular relaxation in response to some endothelium-dependent agonists in vitro. Treatment with cilazapril but not hydralazine for 4 days improved the impaired relaxation of the aorta in response to acetylcholine in spontaneously hypertensive rats (SHR). Thus, ACE inhibitors may improve impaired endothelium-dependent responses in hypertensive animals through an unknown mechanism that does not appear to be related to its effect on blood pressure. 

The second goal of this study was to examine effects of short-term (4 days) treatment with cilazapril, which we expected to have only small effects on systemic arterial pressure, or topical application (40 minutes) of cilazaprilat (the active form of cilazapril) on cerebral vasodilatation in response to both endothelium-dependent and -independent agonists in SHRSP.

Methods

Animals

Male Wistar-Kyoto (WKY) rats (n=46) and SHRSP (n=58) were used in this study. To study long-term effects of cilazapril, 4-month-old WKY rats and SHRSP were divided into four groups. One group of WKY rats and one of SHRSP received tap water. A second group of WKY rats and a second of SHRSP received cilazapril in their drinking water (500 mg/L). This treatment was continued for 3 to 6 months. To study short-term effects of cilazapril, one group of SHRSP, at 9 to 10 months of age, received cilazapril in their drinking water for only 4 days. To study acute effects of the ACE inhibitor (9 to 10 months old), the cranial window was treated with cilazaprilat (10^{-5} M) for 40 minutes before vascular
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, Pco₂ was 42±1 mm Hg, and Po₂ was 76±3 mm Hg in WKY rats; in SHRSP, pH was 7.35±0.01, Pco₂ was 43±2 mm Hg, and Po₂ 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; Pco₂, 35±1 mm Hg; Po₂, 107±4 mm Hg in WKY rats; pH, 7.49±0.01; Pco₂, 34±2 mm Hg; Po₂, 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 craniotomy. Application of vehicle did not affect vessel diameter. With the exception of A23187, the diameter of cerebral arterioles was measured immediately before application of agonists. Responses of cerebral arterioles to two endothelium-independent agonists (nitroglycerin and adenosine) in all study groups. Responses to bradykinin or A23187 in WKY rats (Figs 1 and 2). In contrast, cerebral vasodilatation in response to A23187 was maintained at approximately 38°C. Arterial blood gases and pH were monitored periodically and maintained within normal limits (pH, 7.45±0.01; Pco₂, 35±1 mm Hg; Po₂, 107±4 mm Hg in WKY rats; pH, 7.49±0.01; Pco₂, 34±2 mm Hg; Po₂, 111±2 mm Hg in SHRSP).

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**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.**

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<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial pressure (mm Hg)</th>
<th>Vessel diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=26)</td>
<td>105±4</td>
<td>41±2</td>
</tr>
<tr>
<td>Long-term (n=20)</td>
<td>78±3*</td>
<td>45±2</td>
</tr>
<tr>
<td>Acute (topical) (n=7)</td>
<td>Before</td>
<td>110±4</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>110±6</td>
</tr>
<tr>
<td>SHRSP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=21)</td>
<td>202±5</td>
<td>36±2</td>
</tr>
<tr>
<td>Long-term (n=22)</td>
<td>107±2</td>
<td>39±2</td>
</tr>
<tr>
<td>Short-term (n=7)</td>
<td>157±4</td>
<td>40±2</td>
</tr>
<tr>
<td>Acute (topical) (n=8)</td>
<td>Before</td>
<td>200±6</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>195±4</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats. Values are mean±SEM. Control refers to untreated animals; Long-term refers to treatment with cilazapril for 3 to 6 months; Short-term refers to treatment with cilazapril for 4 days; Topical refers to topical application of cilazaprilat for 40 minutes.

*P<.05 vs control WKY rats.

Cilazapril and cilazaprilat were kindly provided by Hoffmann-La Roche Co, Basel, Switzerland. Bradykinin, A23187, and adenosine were purchased from Sigma Chemical Co, St Louis, Mo. Nitroglycerin was from Du Pont Pharmaceuticals, Wilmington, Del.

**Statistical Analysis**

Comparisons were made between groups of WKY rats and between groups of SHRSP. After analysis of variance, the control group was compared with each of the other treated groups using Duncan’s Multiple-Range Test for multiple comparisons.

**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 cilazaprilat 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 cilazaprilat 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 cilazaprilat increased responses to bradykinin and A23187 in SHRSP (Figs 1 and 2). Topical cilazapril increased cerebral vasodilatation to bradykinin but had no effect on responses to A23187 in WKY rats. Thus, treatment with an ACE inhibitor may improve endothelium-dependent cerebral vasodilatation 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 vasodilatation 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 vasodilatation 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 vasodilatation produced by adenosine ($10^{-4}$ M) in SHRSP. During topical application of cilazaprilat, nitroglycerin produced more vasodilatation in SHRSP than in untreated SHRSP. Thus, treatment with an ACE inhibitor may enhance endothelium-independent cerebral vasodilatation 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-
WKY

Nitroglycerin (M)

<table>
<thead>
<tr>
<th>Diameter, % A</th>
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</thead>
<tbody>
<tr>
<td>35</td>
</tr>
<tr>
<td>50</td>
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</table>

SHRSP

<table>
<thead>
<tr>
<th>Diameter, % A</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
</tr>
<tr>
<td>70</td>
</tr>
</tbody>
</table>

*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).

Endothelium-Dependent Vasodilatation

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

Effects of ACE inhibitors on endothelium-dependent responses have been studied in arteries from normoten-
sive and hypertensive animals.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{9,10}

Acute changes in blood pressure can influence responses of cerebral blood vessels to vasoactive stimuli.\textsuperscript{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...
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 dilatation 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 cilazaprilat 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. Dilatation of pial arterioles in response to nitroprusside and adenosine is not changed after selective injury to endothelial cells. Thus, dilatation 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.

**Acknowledgments**

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