Bradykinin Mediates the Acute Effect of an Angiotensin-Converting Enzyme Inhibitor on Cerebral Autoregulation in Rats

Junichi Takada, MD; Setsuro Ibayashi, MD; Tetsuhiko Nagao, MD; Hiroaki Ooboshi, MD; Takanari Kitazono, MD; Masatoshi Fujishima, MD

Background and Purpose—In patients with stroke and long-standing hypertension, the autoregulation curve of cerebral blood flow (CBF) shifts toward higher blood pressure levels. Angiotensin-converting enzyme (ACE) inhibitors reduce blood pressure and shift the autoregulation curve back to normal in hypertensive patients. ACE inhibitors have 2 major pharmacological properties: they inhibit both the production of angiotensin II and the breakdown of kinins. Hence, we investigated whether the effect of an ACE inhibitor on the lower limit of CBF autoregulation is mediated by the potentiation of bradykinin-mediated vasodilatation.

Methods—In 28 male Sprague-Dawley rats, CBF was measured by laser-Doppler flowmetry during stepwise controlled hypotension. The lower limit of CBF autoregulation was defined as the mean arterial pressure at which CBF decreased by 20% of the baseline value. The rats were treated with an ACE inhibitor, captopril, in the captopril group; a bradykinin BK2-receptor antagonist, Hoe140, in the Hoe140 group; and both agents in the captopril + Hoe140 group. Other rats served as a control group. The lower limits of CBF autoregulation were compared among the 4 groups.

Results—In the captopril group, the lower limit of CBF autoregulation was 43 ± 6 mm Hg (mean ± SD), which was significantly lower than that in the control group (57 ± 14 mm Hg). Inhibition of bradykinin abolished the effect of captopril on the lower limit of CBF autoregulation. Hoe140 alone had no significant effect on the lower limit of CBF autoregulation.

Conclusions—These results suggest that the shift of the lower limit of CBF autoregulation by captopril is mediated, at least in part, by bradykinin. (Stroke. 2001;32:1216-1219.)

Key Words: angiotensin converting enzyme inhibitors ■ autoregulation ■ bradykinin ■ cerebral blood flow

The lower limit of cerebral blood flow (CBF) autoregulation is shifted toward higher blood pressure levels in chronic hypertension; hence, the rapid and excessive reduction of systemic blood pressure potentially leads to a decrease in CBF or, under certain conditions, to brain ischemia. Angiotensin-converting enzyme (ACE) inhibitors, which shift the lower limit of the cerebral autoregulation curve to lower pressure levels, are useful for the treatment of hypertensive patients with cerebrovascular disorders. It has been proposed that such effects of ACE inhibitors on CBF autoregulation are achieved by the attenuation of larger-arterial constriction induced by angiotensin II. On the other hand, ACE inhibitors are capable of inactivating kininase II, a kinin-degrading enzyme, which would result in accumulation of bradykinin. Bradykinin is one of the potent dilators of cerebral arteries, and the effect is mediated by BK2 receptors present on the endothelium. Hoe140 is a selective antagonist against the receptor and has higher potency and longer-lasting antagonism than other antagonistic agents. Thus, we investigated whether the acute effect of the ACE inhibitor captopril on the lower limit of CBF autoregulation is mediated by the potentiation of bradykinin-induced vasodilatation.

Materials and Methods
This study was performed under the control of the Guidelines for Animal Experiments in the Graduate School of Medical Sciences, Kyushu University.

Animal Preparation
Twenty-eight male Sprague-Dawley rats (mean ± SD weight, 390 ± 75 g; aged 2 to 4 months) were used in the present study. Under amobarbital anesthesia (100 mg/kg IP and subsequently 20 mg/kg IV every 1 hour), the femoral arteries on both sides and the right femoral vein were cannulated: one artery for a continuous recording of mean arterial pressure (MAP), the other for controlled bleeding and blood sampling, and the vein for administration of drugs. Depth of anesthesia was evaluated by applying pressure to a paw or the tail and observing changes in heart rate or blood pressure. Additional anesthetic was administered when such changes occurred. The rats were intubated and mounted on a stereotaxic head holder in a sphinx position. Respiration was assisted by a mechanical ventilator (Ro...
dent Ventilator model 683, Harvard Apparatus) with room air and supplemental oxygen. We used a heating pad to keep the rectal temperature constant at 37°C. CBF in the parietal cortex was continuously monitored by laser-Doppler flowmetry (ALF21, Advance Co Ltd) through the burr hole in the skull.8,9 A laser-Doppler probe was placed above the dura mater approximately 4 mm posterior and 2 mm lateral to the bregma.

### Experimental Protocol

The animals were divided into 4 groups (n=7 in each group). In the captopril group, we administered the ACE inhibitor captopril (46 μmol/kg [10 mg/kg] IV) 15 minutes before the reduction of systemic arterial pressure. In the Hoe140 group, the rats were given the bradykinin BK2-receptor antagonist Hoe140 (4 nmol/kg 10 minutes before hemorrhagic hypotension was started, followed by 2 nmol/kg IV every 15 minutes). In the captopril+Hoe140 group, we administered both captopril and Hoe140 according to the schedule described above. In preliminary experiments, we determined the dose of Hoe140 using 7 male Sprague-Dawley rats. First, using 2 rats, we determined a bolus intravenous injection dose of bradykinin (2 μg/kg) that caused transient lowering in MAP by 10 mm Hg. Second, using 5 rats, a dose of Hoe140 (4 nmol/kg) was determined that completely prevented the bradykinin-induced transient hypotension for >20 minutes, and we confirmed that the additional administration of Hoe140 in half of the loading dose every 15 minutes was sufficient to maintain the antagonistic action to bradykinin. Both captopril and Hoe140 were dissolved in saline. All rats received saline as a vehicle, when necessary, to match the intravenous saline volume.

Thirty minutes after stabilization, we started the experimental protocol. Arterial gas parameters were determined at the resting periods (before the administration of captopril and/or Hoe140), before hypotension, and also at the time when MAP was maintained at 40 mm Hg. After the measurement of baseline MAP and CBF, arterial blood was withdrawn from the femoral artery to decrease systemic arterial pressure in a stepwise manner (10 mm Hg per step).9-12 After stabilization of the arterial pressure for at least 3 minutes, CBF was measured at each pressure level. We defined the lower limit of CBF autoregulation as MAP at which CBF decreased by 20% of the baseline value. CBF at each pressure level (every 10 mm Hg step) was expressed as percentage of the baseline value in each rat.

### Results

The major new finding of the present study is that Hoe140, a bradykinin BK2-receptor antagonist, inhibited the captopril-induced change in CBF autoregulation. The result suggests that the modulation of CBF autoregulation by captopril is mediated, at least in part, by bradykinin.

<table>
<thead>
<tr>
<th>TABLE 1. Physiological Variables in 4 Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td><strong>Body weight, g</strong></td>
</tr>
<tr>
<td><strong>Body temperature, °C</strong></td>
</tr>
<tr>
<td><strong>Hematocrit</strong></td>
</tr>
<tr>
<td><strong>At rest</strong></td>
</tr>
<tr>
<td><strong>MAP, mm Hg</strong></td>
</tr>
<tr>
<td><strong>pH</strong></td>
</tr>
<tr>
<td><strong>PaO2, mm Hg</strong></td>
</tr>
<tr>
<td><strong>PaCO2, mm Hg</strong></td>
</tr>
<tr>
<td><strong>After administration of drugs</strong></td>
</tr>
<tr>
<td><strong>MAP, mm Hg</strong></td>
</tr>
<tr>
<td><strong>pH</strong></td>
</tr>
<tr>
<td><strong>PaO2, mm Hg</strong></td>
</tr>
<tr>
<td><strong>PaCO2, mm Hg</strong></td>
</tr>
<tr>
<td>*<em>ΔCBF, %</em></td>
</tr>
</tbody>
</table>

Values are mean±SD.  
*ΔCBF=[(CBF (baseline after administration of drugs)−CBF(at rest))/CBF(at rest)]×100 (%).  
†P<0.002 vs MAP(at rest) in captopril group.  
‡P<0.005 vs MAP(at rest) in captopril+Hoe140 group.

### Statistical Analysis

The results were expressed as mean±SD. Statistical analyses were performed with ANOVA, followed by post hoc Fisher’s protected least significant difference test. P<0.05 was regarded as significant.
Captopril inhibits not only ACE but also kininase II, which catalyzes the breakdown of bradykinin. Bradykinin is one of the potent cerebral vasodilators. Hence, captopril would cause autoregulatory vasodilation by either the inhibition of angiotensin II or the preservation of bradykinin. In the present study the bradykinin receptor blocker inhibited captopril-induced CBF autoregulation. This observation implies that the effect of captopril on the lower limit of CBF autoregulation is mediated by potentiated bradykinin-induced vasodilation. In support of this idea, a previous study demonstrated that acute administration of an ACE inhibitor augmented cerebral vasodilatation to exogenous bradykinin, suggesting that the local kinin-kininase system is operating in cerebral vascular beds. Taken together, it is probable that captopril inhibited the breakdown of endogenous bradykinin, potentiated cerebral vasodilation, and thereby maintained CBF at lower perfusion pressures.

We did not investigate the role of nitric oxide (NO) in bradykinin-induced changes in the lower limit of autoregulation. However, dilator responses of cerebral arteries to bradykinin appear to be mediated by NO in cerebral arteries. Previous studies demonstrated that NO modified the dykinin appear to be mediated by NO in cerebral arter-

The result also argues against the possibility that Hoe140 abolished the shift in the captopril group. The lower limit of autoregulation in the captopril + Hoe140 group was 64±10 mm Hg (double open squares).

In a previous report, in which Hoe140 (0.75 nmol) was infused into the aorta in Sprague-Dawley rats, bradykinin-induced hypotension was still impaired by 71% 1 hour after infusion. In the present intravenous study, the dose of Hoe140 was similar to or even greater than that in the previous study, and it completely prevented the hypotensive effect of bradykinin. In a steady state, Hoe140 concentration in the circulating blood should be constant in the whole body, including cerebral vessels. This means that a sufficient concentration of Hoe140 to block BK2 receptors should have reached cerebral arteries during the experiment. Furthermore, there is no report that the distribution of BK2 receptor is different between endothelial cells (main target of BK2 stimulation) in cerebral and systemic arteries. Therefore, we can reasonably expect that the dose of Hoe140 used in the present study was sufficiently high to inhibit the effect of bradykinin in cerebral arteries.

TABLE 2. MAP Lower Limit of Autoregulation in 4 Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Captopril</th>
<th>Hoe140</th>
<th>Captopril + Hoe140</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>CBF decreased by 20%</td>
<td>57±14</td>
<td>43±8††</td>
<td>69±15</td>
<td>64±10</td>
</tr>
</tbody>
</table>

Values are mean±SD.

†P<0.005 vs control group.
††P<0.0005 vs Hoe140 group.
‡‡P<0.005 vs captopril + Hoe140 group.
of angiotensin II production) on the luminal side of the endothelium. The blood-brain barrier permeability for angiotensin II would be low as well since angiotensin II is octapeptide. Thus, it appears unlikely that angiotensin II produced by ACE on the luminal surface of the endothelium reaches to the adventitial side of the cell across the endothelium-derived relaxing factor(s).

In conclusion, the present results suggest that the effect of acute administration of the ACE inhibitor captopril on the lower limit of CBF autoregulation is mediated, at least in part, by bradykinin.

References


Bradykinin Mediates the Acute Effect of an Angiotensin-Converting Enzyme Inhibitor on Cerebral Autoregulation in Rats
Junichi Takada, Setsuro Ibayashi, Tetsuhiko Nagao, Hiroaki Ooboshi, Takanari Kitazono and Masatoshi Fujishima

Stroke. 2001;32:1216-1219
doi: 10.1161/01.STR.32.5.1216

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/32/5/1216

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/