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±8 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-

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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, Advan
cce Co Ltd) through the burr hole in the skull.7,8 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
at rest). 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 a stepwise manner (10 mm Hg per
10 mm Hg 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. On the assumption that MAP-CBF relationship between 2
adjacent points was linear, we determined MAP at which CBF was
80% of the baseline value. The means of the lower limit of CBF autoregulation thus calculated in each rat were averaged and com-
pared among groups.

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.

Results
Under resting conditions, no significant differences were
found in the physiological variables among the 4 groups (Table 1).
Although captopril reduced MAP from 113±11 to
105±14 mm Hg, resting CBF remained unchanged (Table 1).
MAP was comparable in each group when the bleeding was
started (105±10, 105±14, 107±13, and 101±11 mm Hg in
the control, captopril, Hoe140, and captopril + Hoe140
groups, respectively; P>0.05). Captopril shifted the cerebral
autoregulation curve leftward (Figure 1). The lower limits in the
control group and the captopril group were 57±14 and
43±8 mm Hg, respectively (Table 2). The shift was restored
by Hoe140 (Figure 1). The lower limit in the
captopril + Hoe140 group was 64±10 mm Hg (Table 2).
Hoe140 slightly shifted the lower limit of cerebral autoregu-
lation toward higher pressure levels, although the change in
the lower limit of cerebral autoregulation did not reach statistical
significance (Table 2 and Figure 2).

Discussion
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>
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<tbody>
<tr>
<td>Control (n=7)</td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Body weight, g</td>
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<tr>
<td>Body temperature, °C</td>
</tr>
<tr>
<td>Hematocrit</td>
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<tr>
<td>At rest</td>
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<tr>
<td>MAP, mm Hg</td>
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<tr>
<td>pH</td>
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<tr>
<td>Paco2, mm Hg</td>
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<tr>
<td>Paco2, mm Hg</td>
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<tr>
<td>After administration of drugs</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
</tr>
<tr>
<td>pH</td>
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<tr>
<td>Paco2, mm Hg</td>
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<tr>
<td>Paco2, mm Hg</td>
</tr>
<tr>
<td>ΔCBF, %*</td>
</tr>
</tbody>
</table>

*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.
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 lower limit of cerebral autoregulation. Taken together, NO could be the mediator of the action of ACE inhibitors on CBF autoregulation. However, some investigators failed to demonstrate that the inhibition of NO synthesis changes the lower limit of CBF autoregulation, and others revealed that bradykinin-induced cerebral vasodilatation was mediated by oxygen radicals rather than NO. Therefore, the mediator of the action of ACE inhibitors on CBF autoregulation remains to be determined.

In the present study the lower limit of CBF autoregulation in the Hoe140 group was not significantly higher than that in the control group. This implies that the role of endogenous bradykinin is minor, if any, in the determination of the lower limit of CBF autoregulation under physiological conditions. The result also argues against the possibility that Hoe140 shifts the lower limit of CBF autoregulation rightward in a nonspecific manner.

It has been proposed that ACE inhibitors, by inhibiting angiotensin II production, dilate larger cerebral arteries with compensatory constriction of smaller arteries or arterioles. Such vasoconstriction leads to the augmentation of vasodilatory reserve capacity and thereby shifts the lower limit of autoregulation leftward. However, the notion leaves some room for further discussion. First, the effects of angiotensin II on CBF vary depending on species and source of vessel. For instance, CBF increased in response to intravenously infused exogenous angiotensin II, while infusion of angiotensin II into the internal carotid artery decreased CBF even in the same species of animals (Sprague-Dawley rats). Second, since the blood-brain barrier permeability for captopril is negligible, captopril should exert its main effects (inhibition

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**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>n (n=7)</td>
<td></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<0.05 vs control group. †P<0.0005 vs Hoe140 group. ‡P<0.0005 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 angiotensin II is octapeptide. Thus, it appears unlikely that angiotensin II produced on the luminal side of endothelial cells reaches the adventitial side of the cell across the blood-brain barrier and contracts cerebrovascular smooth muscle.

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


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