Background and Purpose: We investigated the hypothesis that bradykinin generation may induce ischemic brain edema in spontaneously hypertensive rats.

Methods: Cerebral ischemia lasting 3 hours was produced by bilateral common carotid artery occlusion in 67 rats. After the ischemic period, the rats were reperfused. Cerebral water content and energy metabolites (adenosine triphosphate, lactate, and pyruvate), as well as plasma and tissue bradykinin, were measured. Additionally, using the same experimental paradigm, bradykinin synthesis inhibitors (aprotinin [n=7] and soybean trypsin inhibitor [n=7]) were administered immediately after ischemia induction to determine the relation of bradykinin generation to the progression of ischemic brain edema.

Results: Cerebral water content increased during the 3-hour ischemic period, peaked at 30 minutes of reperfusion, and declined thereafter. Bradykinin levels in plasma and tissue rose markedly 30 minutes after reperfusion and fell thereafter. The progressive loss of adenosine triphosphate was mirrored by the rise in lactate. In the treated groups, aprotinin and soybean trypsin inhibitor administration significantly attenuated cerebral edema (p<0.01 and p<0.05, respectively). The treated groups also showed less lactate accumulation and more adenosine triphosphate preservation than did the controls.

Conclusions: These results demonstrate that bradykinin levels in plasma and tissue corresponded to cerebral edema progression and that bradykinin suppression decreased edema formation. These novel findings indicate that bradykinin activation augments the progression of ischemic brain edema. (Stroke 1993;24:571-576)

KEY WORDS • bradykinin • brain edema • cerebral ischemia • rats

The Role of Bradykinin in Mediating Ischemic Brain Edema in Rats

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Bradykinin is known to influence the inflammatory process affecting various tissue reactions, such as peripheral vasodilatation, and to increase vascular permeability. Bradykinin is involved in coagulation, arachidonic acid generation, and the complement, catecholamine, and renin–angiotensin–aldosterone systems. Bradykinin activation can alter the permeability of cerebral vessels during cerebral ischemia and may therefore influence the progression of ischemic edema. The role of bradykinin in the pathogenesis of ischemic brain edema, however, is not yet clearly understood. Recently, activation of the kallikrein–kinin system in vasogenic brain edema has been reported.

Similar involvement of the kallikrein–kinin system in cerebral edema progression may also occur in the ischemic brain. To investigate this question, we have determined the relation between ischemic edema progression and endogenously generated bradykinin in spontaneously hypertensive rats subjected to 3 hours of ischemia followed by recirculation. To further evaluate the effect of endogenously generated bradykinin on ischemic edema progression, we studied the effect of aprotinin or soybean trypsin inhibitor, compounds that specifically inhibit the kallikrein–kinin system, on cerebral edema and energy metabolism (adenosine triphosphate [ATP], lactate, and pyruvate) in rats subjected to the same experimental conditions.

Materials and Methods

Cerebral ischemia was produced in 67 16-week-old, 300-g, male spontaneously hypertensive rats (Hoshino Laboratory Animals, Saitama, Japan). The rats were anesthetized with 2% halothane in a mixture of 70% nitrogen/30% oxygen. The femoral artery and vein were catheterized to permit blood pressure monitoring (Nihon Kohden TP 400T) and intravenous drug infusion. Aneurysm clips were placed on both common carotid arteries through a midline approach. After occlusion for 3 hours, the clips were removed to permit recirculation. At 0, 30, and 60 minutes after reperfusion, the rats were reanesthetized and killed by microwave application to the head (Toshiba microwave applicator, Tokyo; 5-kW output for 1.5 seconds); this immediately inactivated enzyme activity in the brain. The brains were then removed, and the portion of the forebrain anterior to the bregma was quickly dissected to permit analysis of tissue bradykinin, cerebral water content, and cerebral metabolites (ATP, lactate, and pyruvate). The dissected
tissue was divided into two parts: an anterior part used for determination of tissue bradykinin and brain water content and a posterior part used for assay of brain metabolites. Plasma bradykinin levels were measured in blood samples collected from the external jugular vein just before microwave application.

Using this paradigm, we investigated the effects of two bradykinin synthesis inhibitors on brain edema and brain energy metabolism after 30 minutes of reperfusion following 3 hours of ischemia. The rats received either 66,000 units/kg aprotinin (Bayer Chemical Co., Osaka, Japan) (*n* = 7), or 20 mg/kg soybean trypsin inhibitor (P-L Biochemicals, Inc., Milwaukee, Wis.) (*n* = 7); a saline infusion was administered to controls (*n* = 7). The drugs were administered into the femoral vein immediately after ischemia induction.

To measure brain bradykinin levels, the blood samples were mixed with the kallikrein inhibitors Trasylol (6,000 units; Union Chemical Co., Tokyo) and soybean trypsin inhibitor (2 mg; CalBiochem-Novabiochem Co., San Diego, Calif.) and with the kininase inhibitors ethylenediaminetetraacetate (EDTA) (20 mg; Nacalai Tesque, Inc., Kyoto, Japan) and protamine (5 mg; Wako Pure Chemical Industries, Ltd., Osaka, Japan). They were then centrifuged at 3,000 rpm for 5 minutes. The supernatant was collected and kept frozen at −70°C until assayed.

To measure brain tissue levels of bradykinin, the collected brain samples were homogenized in a Potter-type homogenizer in 4 ml 0.1 M acetic acid at 4°C. The homogenate was then adjusted to 10 ml by diluting it with 0.1 M acetic acid and sodium chloride (3.5 g). This homogenate was mixed thoroughly, allowed to stand in the ice-cold water, and then centrifuged for 10 minutes (3,000 rpm, 4°C). Trasylol, soybean trypsin inhibitor, EDTA, and protamine were added to the supernatant in the same amounts as in the plasma, and the supernatant was then frozen at −70°C until assayed.

The supernatant and plasma samples were deproteinized by mixing with isopropyl alcohol; to remove lipids, the samples were mixed with petroleum ether. After deproteinization and lipid removal, the samples were condensed in nitrogen, and polyethylene glycol was added for bound/free separation. Radioimmunoassay was performed on these samples for bradykinin measurement. These values are expressed as picograms per milliliter in blood, and nanograms per gram for brain tissue.

Cerebral water content was measured by the freeze-dry method. The rat brains were removed from the skull immediately after decapitation and dissected as described above, then quickly weighed to determine the wet weight. The brain tissue was freeze-dried for 72 hours. Dry weight was then measured, and the brain water content was calculated. Brain water content was expressed as percentage of brain wet weight.

The brains used to measure energy metabolite levels were subjected to microwave irradiation before they were dissected from the skull. They were weighed and homogenized in 5 ml 6% trichloroacetic acid with a poltron homogenizer. After 20 minutes of centrifugation (3,000 rpm, 4°C), the supernatant was stored at −20°C until energy metabolite analysis. ATP concentrations were measured by luciferin–luciferase bioluminescence. Lactate and pyruvate values were determined by the lactate dehydrogenase enzymatic method.

All values obtained from the above studies are expressed as mean±SD. The statistical significance of comparisons between groups was evaluated by analysis of variance followed by Scheffe's *F* test.

### Results

The mean arterial blood pressure (MABP) before ischemia was 183±7 mm Hg in the untreated control animals and 184±6 mm Hg and 179±7 mm Hg in the aprotinin- and soybean trypsin inhibitor–treated rats, respectively. At 3-hour occlusion, MABP had risen to 226±11 mm Hg, 212±12 mm Hg, and 206±12 mm Hg in the control, aprotinin-, and soybean trypsin inhibitor–treated groups, respectively. MABP fell substantially just after recirculation, followed by a gradual rise to 159±14 mm Hg, 150±12 mm Hg, and 150±11 mm Hg after 30 minutes of recirculation in the control, aprotinin-, and soybean trypsin inhibitor–treated groups, respectively. No significant differences in MABP were found among the groups before, during, and after ischemia.

The cerebral water content and the tissue and plasma bradykinin levels all followed the same temporal pattern; i.e., they rose by 3 hours of ischemia, reached their highest level 30 minutes after recirculation, and fell by 60 minutes of recirculation (Figure 1, Table 1). Lactate values also followed the same temporal pattern, although the progressive decrease in ATP was mirrored by the rise in lactate (Figure 2, upper and middle panels; Table 1). Pyruvate levels did not show a significant change throughout the experiment (Figure 2, lower panel; Table 1).

In the aprotinin-treated group, the brain water content was significantly lower than that in the control group (*p*<0.01; Table 2), and drug administration significantly suppressed the production of tissue bradykinin (*p*<0.01; Table 2). Furthermore, this group showed significantly lower lactate accumulation and more preservation of ATP concentration than was seen in the controls (*p*<0.01 and *p*<0.05, respectively; Table 2). In the soybean trypsin inhibitor–treated group, the brain water content was also significantly lower than it was in the control group (*p*<0.05; Table 2). Treatment with this drug also suppressed bradykinin production significantly (*p*<0.01; Table 2). There was lower lactate accumulation and more preservation of ATP concentration in this group compared with the controls; however, these differences were not statistically significant (Table 2). Pyruvate levels in both experimental groups failed to show a significant change compared with the controls (Table 2).

### Discussion

The present study delineates the time course of cerebral edema and changes in energy metabolism in the postischemic brain. In our studies, the level of brain edema closely reflected bradykinin level changes in both plasma and tissue (Figure 1). The brain water content increased during 3 hours of ischemia. The water content peaked 30 minutes after recirculation, then returned to normal within the next 30 minutes of recirculation. The tissue and plasma bradykinin levels showed a similar temporal pattern; however, the plasma bradykinin levels...
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rose significantly at 3 hours of ischemia, whereas the tissue levels did not. To confirm further the relation between bradykinin and cerebral edema, we evaluated the effects of the bradykinin synthesis inhibitors aprotinin and soybean trypsin inhibitor in rats subjected to bilateral common carotid artery occlusion. The results showed that the intravenous administration of aprotinin or soybean trypsin inhibitor prevented the progression of ischemic brain edema. Taken together, our results demonstrated that bradykinin levels corresponded closely to the development of brain edema and that edema formation decreased in rats whose bradykinin generation was suppressed.

Bradykinin is generated by activation of the kallikrein-kinin system and is known to be an important chemical mediator of local inflammation. It is also known to increase peripheral vasodilatation and vascular permeability\textsuperscript{1–5} and has been reported to affect blood vessels and vascular smooth muscle.\textsuperscript{12,13} When administered intravenously, bradykinin has been shown to increase systemic blood pressure.\textsuperscript{14,15} In inflamed tissue, bradykinin has been found to potentiate fluid exudation from the postcapillary venules into the extracellular space.\textsuperscript{16} In ischemic brain edema, especially in the vasogenic phase, fluid accumulation in the extracellular space resembles fluid accumulation produced in inflamed tissues. Thus, the suspicion arose that a similar mechanism may influence the production of ischemic brain edema. Recently, the role of the kallikrein–kinin system in the production of vasogenic brain edema has been investigated.\textsuperscript{8–10} Therefore, we investigated the hypothesis that bradykinin generation may induce ischemic brain edema in spontaneously hypertensive rats.

We had previously examined the effect of exogenously administered bradykinin on cerebral edema. Our results showed that retrograde infusion of bradykinin into the internal carotid artery through the external carotid artery produced edema in the infused region but not the contralateral hemisphere. Using the horseradish peroxidase tracer method, we observed an increase of vascular permeability with an increase of pinocytosis in the perfused region by electron microscopy.\textsuperscript{17} Considering the similar time course of changes in tissue, plasma bradykinin, and edema progression, this finding suggests that through its effect on cerebral vascular permeability, bradykinin may influence cerebral edema development.

**Table 1.** Brain Water Content, Plasma Bradykinin Levels, Tissue Bradykinin Levels, and Energy Metabolites in Spontaneously Hypertensive Rats Subjected to 3 Hours of Ischemia Followed by Recirculation

<table>
<thead>
<tr>
<th>Determination</th>
<th>Control (n=6)</th>
<th>Period of postischemic recirculation (min)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 (n=6)</td>
<td>30 (n=6)</td>
<td>60 (n=5)</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>78.01±0.20</td>
<td>79.20±0.49</td>
<td>80.69±0.75*</td>
<td>78.95±1.10</td>
</tr>
<tr>
<td>Plasma bradykinin (pg/ml)</td>
<td>74.0±14.9</td>
<td>169.8±30.6</td>
<td>486.7±136.1*</td>
<td>166.0±31.9</td>
</tr>
<tr>
<td>Tissue bradykinin (ng/g brain)</td>
<td>4.45±1.66</td>
<td>4.66±0.61</td>
<td>13.67±3.95*</td>
<td>5.19±1.82</td>
</tr>
<tr>
<td>Energy metabolites (μmol/g brain)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>2.64±0.11</td>
<td>0.14±0.08*</td>
<td>0.18±0.09*</td>
<td>0.75±0.48*</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.38±0.28</td>
<td>32.38±7.16*</td>
<td>39.39±7.99*</td>
<td>11.01±7.38</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.18±0.06</td>
<td>0.12±0.07</td>
<td>0.18±0.02</td>
<td>0.26±0.18</td>
</tr>
</tbody>
</table>

Values are mean±SD for number of animals in parentheses. ATP, adenosine triphosphate. Brain water content is expressed as percentage of whole tissue weight.

*p<0.01 compared with control rats by analysis of variance followed by Scheffe's F test.
We used microwave irradiation to measure brain water content and brain energy metabolites in the same brain sample because we could not estimate the correct relation between brain edema and metabolism in different rat brains. To estimate the effect of microwave irradiation on brain water content, we measured brain water content with and without microwave irradiation (data not shown). According to our results, brain water content with microwave irradiation was a little lower than without microwave irradiation (approximately 0.57–0.71%). We could thus measure brain water content and energy metabolites using the same microwave-irradiated brain. We were concerned that with bilateral common carotid arteries occlusion, ischemia would be more severe in the frontal than in the occipital region of the brain due to the intact posterior and basilar circulation; we therefore chose the part of the brain anterior to the bregma to make our measurement. The portion of the brain anterior to the bregma could thus be more homogenously affected by bilateral common carotid artery occlusion. However, it is possible that the measurement of brain water content in the anterior part of the brain anterior to the bregma and that of brain energy metabolites in the posterior part of the same brain may represent changes resulting from different degrees of ischemia, although the results showed that cerebral edema progression corresponded to energy metabolism impairment.

Interestingly, cerebral edema as represented by brain water content was highest 30 minutes after recirculation, corresponding to plasma and tissue bradykinin levels (Figure 1). Tissue lactate levels were also highest 30 minutes after recirculation. It can be postulated that mitochondrial dysfunction and glucose supply resumed after recirculation elevated the lactate levels.

Because changes in cerebral tissue bradykinin levels correlated well with plasma levels, it is possible that 1) bradykinin was generated from the cerebral tissue, and plasma levels reflected bradykinin generation in the ischemic brain tissue, or that 2) elevated bradykinin levels in tissue reflected the preferential generation of bradykinin in the blood vessels. However, further studies are needed to confirm the origin of bradykinin.

Additionally, changes in energy metabolism in the treated groups were analyzed. Results showed that treatment with aprotinin improved metabolite levels significantly, whereas treatment with soybean trypsin inhibitor did not. There was, however, a tendency toward improved energy metabolism in the soybean trypsin inhibitor groups. The different outcomes may be attributed to the fact that aprotinin, a polyvalent protease inhibitor, exhibits its inhibitory action on both tissue and plasma kallikrein, whereas soybean trypsin inhibitor preferentially antagonizes plasma kallikrein\textsuperscript{18}; however, another possibility is that the inhibitors affect intravascular clotting differently.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Water content (%)</th>
<th>Tissue bradykinin (ng/g brain)</th>
<th>Metabolites (μmol/g brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATP</td>
<td>Lactate</td>
</tr>
<tr>
<td>Saline</td>
<td>7</td>
<td>80.34±0.94</td>
<td>11.95±3.62</td>
<td>0.41±0.14</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>7</td>
<td>77.79±0.21</td>
<td>2.82±0.21</td>
<td>2.38±1.60\textsuperscript{t}</td>
</tr>
<tr>
<td>Soybean trypsin inhibitor</td>
<td>7</td>
<td>79.22±0.83\textsuperscript{*}</td>
<td>6.91±1.66\textsuperscript{t}</td>
<td>0.91±0.36</td>
</tr>
</tbody>
</table>

Values are mean±SD. n, number of animals. ATP, adenosine triphosphate.

\textsuperscript{*}p<0.05, \textsuperscript{t}p<0.01 compared with control rats by analysis of variance followed by Scheffe’s F test.
It was recently reported that prostaglandin synthesis and leukotriene synthesis were increased during the recirculation period after an ischemic insult. However, the leukotriene synthesis were increased during the recirculation period after an ischemic insult. Therefore, it can be hypothesized that bradykinin elevates phospholipase A2, thereby accelerating eicosanoid production leading to increased ischemic cerebral edema. Our results, which show that the progression of edema correlated closely to the rise in plasma and tissue bradykinin levels (i.e., brain edema was most severe during the peak elevation of bradykinin levels), support this hypothesis. This speculation is also consistent with reports that prostaglandin synthesis and leukotriene synthesis were increased during the recirculation period after an ischemic insult. However, the exact sequence of events remains to be investigated.

In conclusion, these novel findings indicate that activation of bradykinin augments the progression of ischemic brain edema, and endogenously produced bradykinin may have an important role in aggravating ischemic brain edema.

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References
brain bradykinin levels, ATP loss, and lactate accumulation. Soybean trypsin inhibitor, which was not as effective as aprotinin in reducing brain bradykinin levels, did not significantly reduce ATP loss or lactate accumulation.

Both aprotinin and soybean trypsin inhibitor are attractive therapeutic candidates for testing in acute human stroke for several reasons. First, plasma bradykinin levels can be monitored to determine whether the treatments are effective in blocking the kallikrein–kinin system. Second, much of the brain bradykinin increase may come from peripheral sources. Therefore, these drugs may not need to penetrate the blood–brain barrier to have a therapeutic benefit. Third, inhibitors of the kallikrein–kinin system may well have synergistic effects with other potentially neuroprotective drugs aimed at specific neuronal secondary injury mechanisms, such as nimodipine, tirilazad mesylate, and MK-801.

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The role of bradykinin in mediating ischemic brain edema in rats.

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