Ischemic Brain Edema Following Occlusion of the Middle Cerebral Artery in the Rat. II: Alteration of the Eicosanoid Synthesis Profile of Brain Microvessels


SUMMARY Using the rat middle cerebral artery occlusion model, alterations in the eicosanoid synthetic capacity of brain microvessels following ischemia were studied by radiochromatography. Brain microvessels of normal rats predominantly produced hydroxyacids with relatively small amounts of PGD₂ and PGE₂ from exogenous arachidonic acid. Confirmation that hydroxyacids and prostaglandins were products respectively of lipoxygenase(s) and cyclooxygenase was obtained by experiments using indomethacin and eicosatetraynoic acid. The eicosanoid synthetic capacity of the brain microvessel, especially of hydroxyacids, was significantly enhanced 24 and 72 hours after the onset of ischemia. Because this is the phase of maximum edema in the present model, enhanced eicosanoid production in the brain microvessel may be involved in the mechanisms that underly ischemic brain edema.

Stroke Vol 16, No 1, 1985
crose, 5 mM-EDTA, and 50 mM-Tris HCl (pH 7.3). Then, the suspension was serially passed through nylon meshes having pore sizes of 670 μm and 335 μm, respectively. The filtrate was centrifuged at 3,500 g for 10 minutes, and the resultant precipitate was resuspended into the same buffer. Each 2.5 ml were layered on a discontinuous density gradient consisting of 1.0 M–, 1.3 M–, and 1.5 M-sucrose (3 ml each). Then the gradient was centrifuged at 58,000 g for 60 minutes. The subfraction at the bottom of the tube were collected and resuspended in the above-described buffer.

### Metabolism of [14C]-arachidonic Acid by the Rat Brain MicrovesSEL

The metabolism of [14C]-arachidonic acid by the rat brain microvesSEL was studied within the day of preparation as follows. The microvesSEL (40–50 μg protein) was preincubated for 10 minutes at 37°C in 0.1 ml of 50 mM-Tris HCl (pH 7.4) containing 10.0 mM-glucose with or without drugs. After preincubation, 50 μl of [14C]-arachidonic acid (0.2 μCi; final concentration of 6.5 μM) were added to the incubation mixture, which was incubated for an additional 60 minutes at 37°C in the dark without agitation. The reaction was terminated by chilling, followed by quickly adding an appropriate amount of 1 N-HCl to bring the pH of the reaction mixture to 3.0. Then the mixture was extracted two times with 8 ml of ethyl acetate. The resulting organic phase was evaporated to dryness with a stream of nitrogen. The residue was then dissolved in a small amount of ethanol and applied to a precoated silica gel thin-layer plate (60F 254, Merck). Prostaglandins D2, E2, 6-keto-PGF1α (Ran Biochem), arachidonic acid (Sigma), and [14C]-TXB2 (New England Nuclear, Boston, Mass.) were also applied to the plate. The plate was developed in ethyl acetate-isooctane-acetic acid-water (11:5:2:10, organic phase). The plate was dried and scanned for its radioactivities by a radiochromatogram-scanner (TRM-1B, Aloka). Nonradioactive arachidonic acid and prostaglandin markers were visualized by spraying with phosphomolybdic acid in ethanol. The areas corresponding to authentic standard arachidonic acid, PGD2, PGE2, and 6-keto-PGF1α were scraped off the plate directly into scintillation vials as was the remaining silica gel in each lane. Ten milliliters of scintillating fluid (Aquasol-2, New England Nuclear) were added, and the radioactivity in each sample was measured in a Beckman LS-150 liquid scintillation counter. The radioactivity in each zone was expressed as the percentage of the total counts per minute for the entire lane. Throughout this experiment, microvessels incubated for 10 minutes in boiling water were used as a blank. The numbers of rats used for the examination of the arachidonate metabolism of the brain microvesSEL were 64, 48, 224, and 128 for each experimental group of normal, 24 hours after sham-operation, 24 hours after MCA occlusion, and 72 hours after MCA occlusion, respectively.

### Results

#### Eicosanoid Synthetic Capacity of the Rat Brain MicrovesSEL

A typical thin-layer chromatography pattern of eicosanoid production of the microvessels obtained from the normal and MCA-occluded rats (24 hours postoperatively) is shown in figure 1. The percentage conversion of [14C]-arachidonic acid is summarized in table 1. From these results, it became apparent that normal rat microvessels predominantly produced hydroxyacids (Rf 0.51) and PGE2 (Rf 0.30). In the case of MCA-occluded rats (24 and 72 hours postoperatively), on the other hand, all the eicosanoids were shown to be markedly increased.

The addition of indomethacin to the incubation mixture caused a significant reduction of only the cyclooxygenase products, whereas ETYA significantly reduced all the products (table 2). Thus indomethacin did not significantly reduce lipoxygenase products (hydroxyacids), but ETYA did.

#### Discussion

Fatty acids comprising membrane lipids are liberated and accumulate in the brain following ischemia. Although most of these free fatty acids are considered to be washed out of the brain or reacylated, conversion of arachidonic acid to prostaglandins via the arachidionate cascade has been shown to occur in various models. Because of the potent biological effects of prostaglandins, it has been suspected that the arachidonate cascade is involved in the genesis of ischemic brain edema.

The role of prostaglandins in brain edema has been
The present study revealed that the cerebral microvessel of normal rats has the capacity to convert exogenous arachidonic acid to various prostaglandins and hydroxy (hydroperoxy) acids. The percent conversion of \([1^4\text{C}]\)-arachidonic acid to each product was in agreement with the result of Geese et al.\(^2\) Further, the eicosanoid synthetic capacity of the brain microvessel was shown to be significantly enhanced following MCA occlusion. The study using ETYA and indomethacin (table 2) indicates that prostaglandins and hydroxyacids detected by the present method are products of cyclooxygenase and lipoxygenase, respectively.\(^2\) Therefore, the present study suggests that the activities of both enzymes in the brain microvessel were enhanced following MCA occlusion. As this occurred during the period of 24–72 hours after MCA occlusion when the edema development was most rapid in the present model,\(^1\) it is tempting to speculate that the enhanced eicosanoid synthesis in the brain microvessel is causally related to edema formation.

As with the results obtained with other models, the administration of indomethacin in the present model did not effect beneficially brain edema (unpublished data). Considering these results, cyclooxygenase products may not play a major role in the occurrence of brain edema also in the present model, although the measurement of each prostaglandin in situ is required before a conclusion is reached.

On the other hand, little has been known about the lipoxygenase activity or the kinds and actions of lipoygenase products in the brain or the brain microvessel. In organs other than the brain, however, there is evidence that lipoygenase products increase the vessel permeability. Leukotrienes C\(_4\) and D\(_4\), which are lipoygenase products, were shown to increase plasma exudation when injected into the skin.\(^2\) In the perfused lung, lipoygenase products enhanced transalveolar exudation.\(^3\) Also, the inhibition of lipoxygenase activity by BW755C was shown to mitigate the inflammatory response of the ischemic myocardium.\(^4\) These results together with the present data showing that the lipoxygenase activity of the microvessel was significantly enhanced concomitant to the edema development raise the possibility that the products of lipoygenase rather than of cyclooxygenase are relevant to the pathogenetic mechanism underlying ischemic brain edema. In support of this view, we have previously observed that the intrathecal injection of 15-hydroperoxy-arachidonic acid(15-HPAA), a plant lipoygenase product, caused prolonged vasospasm of the basilar artery in dogs associated with pronounced degenerative changes in the arterial endothelium.\(^5\)

Insomuch as the present study revealed that the enhancement of the eicosanoid synthetic capacity of the brain microvessel paralleled the development of brain edema following MCA occlusion in rats, their relationship obviously requires further elucidation. Among

# Table 1: Eicosanoid Profiles of The Rat Brain Microvessel

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hydroxyacids</th>
<th>PGD(_2)</th>
<th>PGF(_2\alpha)</th>
<th>TxB(_2)</th>
<th>6-keto-PGF(_{1\alpha})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (24)</td>
<td>3.24 ± 0.22</td>
<td>1.21 ± 0.18</td>
<td>0.98 ± 0.26</td>
<td>0.38 ± 0.04</td>
<td>0.73 ± 0.21</td>
</tr>
<tr>
<td>24 hr-sham (9)</td>
<td>3.15 ± 0.37</td>
<td>1.28 ± 0.20</td>
<td>0.93 ± 0.16</td>
<td>0.44 ± 0.08</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>24 hr-MCA (44)</td>
<td>8.83 ± 0.88(\dagger)</td>
<td>2.63 ± 0.31(*))</td>
<td>2.08 ± 0.26(\dagger)</td>
<td>1.59 ± 0.22(\dagger)</td>
<td>1.68 ± 0.27(\dagger)</td>
</tr>
<tr>
<td>72 hr-MCA (24)</td>
<td>6.43 ± 0.43(\dagger)</td>
<td>4.72 ± 0.66(\dagger)</td>
<td>2.35 ± 0.31(\dagger)</td>
<td>1.14 ± 0.18(\dagger)</td>
<td>1.88 ± 0.20(\dagger)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of experiments.
Normal vs MCA at \(^*p < 0.05; ^\dagger p < 0.01; ^\ddagger p < 0.001.\) (Student's t-test.)

The eicosanoid profiles of the rat brain microvessel are shown as percentage conversion of \([1^4\text{C}]\)-arachidonic acid. Normal: Normal rats; 24 hr-sham: rats sacrificed 24 hours after the sham operation; 24 hr-MCA: rats sacrificed 24 hours after the MCA occlusion; 72 hr-MCA: rats sacrificed 72 hours after the MCA occlusion. The difference from the normal control values was statistically examined using Student's t-test.
TABLE 2  
Effects of Indomethacin and ETYA on The Eicosanoid Synthesis of The Brain Microvessel

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hydroxycids</th>
<th>PGD₂</th>
<th>PGE₂</th>
<th>TxB₂</th>
<th>PGF₁₀</th>
<th>6-keto-PGF₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (24)</td>
<td>3.24±0.22</td>
<td>1.21±0.18</td>
<td>0.98±0.26</td>
<td>0.38±0.04</td>
<td>0.73±0.21</td>
<td>0.50±0.07</td>
</tr>
<tr>
<td>Indomethacin (1 × 10⁻⁵M) (4)</td>
<td>4.22±0.60</td>
<td>0.26±0.07*</td>
<td>0.87±0.08</td>
<td>0.17±0.03</td>
<td>0.23±0.05</td>
<td>0.25±0.04</td>
</tr>
<tr>
<td>Indomethacin (1 × 10⁻⁵M) (4)</td>
<td>3.68±0.47</td>
<td>0.20±0.05*</td>
<td>0.18±0.04†</td>
<td>0.06±0.01†</td>
<td>0.11±0.01*</td>
<td>0.13±0.02*</td>
</tr>
<tr>
<td>ETYA (3 × 10⁻⁴M) (4)</td>
<td>1.99±0.41*</td>
<td>0.23±0.05*</td>
<td>0.32±0.08</td>
<td>0.18±0.03</td>
<td>0.19±0.05</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>ETYA (3 × 10⁻⁴M) (4)</td>
<td>1.40±0.27†</td>
<td>0.10±0.02*</td>
<td>0.17±0.02*</td>
<td>0.06±0.02†</td>
<td>0.13±0.02</td>
<td>0.13±0.00*</td>
</tr>
</tbody>
</table>

*p < 0.05, †p < 0.01.
Effects of indomethacin and ETYA on the eicosanoid synthesis of the brain microvessel obtained from normal rats are shown. Statistical analysis was undertaken using Student’s t-test.

Acknowledgments
The authors wish to thank Dr. Graham M Teasdale, University of Glasgow, for his formative discussions, and Miss Mikiko Sonobe for typing the manuscript.

References
Ischemic brain edema following occlusion of the middle cerebral artery in the rat. II: Alteration of the eicosanoid synthesis profile of brain microvessels.
T Asano, O Gotoh, T Koide and K Takakura

Stroke. 1985;16:110-113
doi: 10.1161/01.STR.16.1.110

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/16/1/110

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/