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 produced respectively of lipoygenase(s) and cyclooxygenase was obtained by experiments using indomethacin and eicosatetraenoic 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.

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BREAKDOWN of the blood-brain barrier (BBB) to plasma solutes such as proteins and electrolytes constitutes an essential mechanism underlying ischemic brain edema. In agreement with previous reports,1-4 our studies of the rat middle cerebral artery (MCA) occlusion model revealed that there was a close correlation between the increases in the brain water and sodium contents throughout the development of ischemic brain edema.5 Therefore, from a therapeutic point of view, it seems of utmost importance to identify those chemical factors that might affect the BBB permeability following ischemia.

Several compounds, such as cyclic AMP, biogenic amines, and the kallikrein-kinogen-kinin system, have been suggested to be possible candidates that might cause BBB permeability changes.6 7 However, with each of these compounds, inconsistent data have been reported.7 8 In this respect, the hypothesis suggested by Demopoulos et al9 seems of interest. They assumed that in the brain rendered ischemic, active oxygens are generated and these cause membrane disruption due to propagation of free radical reactions. This represents a form of lipid peroxidation due to non-enzymatic reactions. On the other hand, it was recently shown that another form of lipid peroxidation due to enzymatic reactions, namely, the arachidonate cascade, exists in the brain. Free arachidonic acid accumulates following ischemia,10-12 and at least part of it is peroxidized via the arachidonate cascade.13-15 Cyclooxygenase and lipoxygenase products, such as prostaglandins and leukotrienes, are known to possess multiple biological actions, which may be related to the occurrence of brain edema.16 17 In addition to these products, an oxidant, currently deemed as hydroxyl radical, is liberated in the conversion of PGG2 to PGH2.18 19 Hydroperoxides (HPETEs) which can act as free radicals or may release more potent oxidants through the action of the hydroperoxidase associated with PG synthetase20 are produced by lipoxygenases. Therefore, when the role of free radicals in ischemic brain edema is considered, it seems indispensable to investigate the activity of the arachidonate cascade following ischemia.

In the present study, the eicosanoid synthetic capacity of the cerebral microvessel obtained from hemispheres of normal or MCA-occluded rats was examined. Brain microvessels were specifically studied because they are reportedly rich in the enzymes comprising the arachidonate cascade,20-22 and, mostly being parenchymal small vessels, it constitutes essential parts of the cerebral microcirculation as well as being the site of the BBB. The unilateral MCA occlusion in the rat was used as the model of cerebral ischemia, because it was shown to provide a well-reproducible pattern of edema development.5

Materials and Methods

Preparation of the Rat Brain Microvessel

Male Sprague-Dawley rats, weighing 250-300 g were subjected to unilateral MCA occlusion by the method described elsewhere.5 Sixteen rats were successively operated on in a day, and they were sacrificed 24 and 72 hours later. Following brain perfusion with 100 ml of physiological saline from the heart at a pressure of 120 mmHg, the brain was rapidly removed and divided into its two hemispheres. The 16 hemispheres of the MCA-occluded or sham-operated side were assembled and used to prepare the brain microvessel. Such a procedure was repeated three to four times for each experimental group.

Preparation of the brain microvessel was carried out according to the method of Gesce et al22 with minor modifications. All steps in the procedure were carried out in the cold (0-4°C). Briefly, each hemisphere was chopped with scissors in 10 volumes of 0.25 M-su-
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 twice 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 D\textsubscript{2}, E\textsubscript{2}, 6-keto-PGF\textsubscript{1α}, (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 radiochromatography 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, PGD\textsubscript{2}, PGE\textsubscript{2}, and 6-keto-PGF\textsubscript{1α} 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.

Effects of Indomethacin and Eicosatetraynoic Acid on the Metabolism of [14C]-arachidonic Acid of the Rat Brain Microvessel

To determine whether the eicosanoids produced by the brain microvessel are products of the arachidonate cascade, the normal microvessel was incubated with [14C]-AA in the presence of indomethacin (10\textsuperscript{-4} and 10\textsuperscript{-5} M) or eicosatetraynoic acid (ETYA; 3 × 10\textsuperscript{-4} and 3 × 10\textsuperscript{-3} M). Thirty-two rats were used for each experiment.

Statistical Analysis

Values are expressed as means ± SEM. The statistical significance of differences between group means was evaluated by Student’s unpaired two-tail t-test. A p value of less than 0.05 was considered significant.

Protein Estimation

The protein concentration of the rat brain microvesel suspension was determined according to the method of Lowry et al\textsuperscript{19} with crystalline bovine serum albumin as standard.

Chemicals

ETYA was a kind gift from Nippon Roche Research Center. Indomethacin was purchased from Sigma (St. Louis, Mo.).

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 PGE\textsubscript{2} (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.\textsuperscript{10-12} 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 arachidone cascade\textsuperscript{13-15} and/or to unidentified peroxides\textsuperscript{11} 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
studied in experiments in which indomethacin was used as an inhibitor of cyclooxygenase. These studies consisted of finding evidence of a beneficial effect, suggesting that cyclooxygenase products are not major factors in the production of edema. However, as indicated by a previous report and the present result (table 2), indomethacin in the dose range used in those experiments probably does not inhibit lipoxygenases. In addition, it is known that indomethacin increases the availability of arachidonic acid to lipoxygenases. Therefore, even if the cyclooxygenase pathway may not be involved in the occurrence of ischemic brain edema, the role of the lipoxygenase pathway needs to be clarified.

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 [14C]-arachidonic acid to each product was in good agreement with the result of Geese et al.22 For vessel of normal rats (solid line) and MCA-occluded rats (24 hours after the MCA occlusion: broken line) are shown. The Rf value of each peak is indicated at the bottom of the figure.

TABLE 1  Eicosanoid Profiles of The Rat Brain Microvessel

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hydroxyacids</th>
<th>PGD₂</th>
<th>PGE₂</th>
<th>TxB₂</th>
<th>6-keto-PGF₁₀</th>
<th>5-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 ± 0.06</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>
<td>0.44 ± 0.07</td>
</tr>
<tr>
<td>24 hr-MCA (44)</td>
<td>8.83 ± 0.88‡</td>
<td>2.63 ± 0.31*</td>
<td>2.08 ± 0.26†</td>
<td>1.59 ± 0.22*</td>
<td>1.68 ± 0.27*</td>
<td>1.44 ± 0.26*</td>
</tr>
<tr>
<td>72 hr-MCA (24)</td>
<td>6.43 ± 0.43‡</td>
<td>4.72 ± 0.66‡</td>
<td>2.35 ± 0.31†</td>
<td>1.14 ± 0.18†</td>
<td>1.88 ± 0.20†</td>
<td>1.46 ± 0.19†</td>
</tr>
</tbody>
</table>

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

The eicosanoid profiles of the rat brain microvessel are shown as percentage conversion of [14C]-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.
various eicosanoids, the role of lipoygenase products which were shown to be the major eicosanoid products of the brain microvessel in the present study, would merit further investigation.

Acknowledgments

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References


### Table 2

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Hydroxyacids</th>
<th>PGD$_2$</th>
<th>PGE$_2$</th>
<th>TXB$_2$</th>
<th>PGF$_{20}$</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>
<td>0.50±0.07</td>
</tr>
<tr>
<td>Indomethacin</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>(1 x 10$^{-5}$M) (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 x 10$^{-4}$M) (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>(3 x 10$^{-3}$M)</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.
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