Regional Differences in Rat Brain Lipids During Global Ischemia

Mikulas Chavko, PhD, and Edwin M. Nemoto, PhD

Background and Purpose: Membrane lipid degradation plays an important role in the pathogenesis of ischemic brain damage, but there is little information on changes in cerebrosides, sulfatides, and sphingomyelin. We studied regional changes in the quantities of these lipids during complete global brain ischemia in rats.

Methods: Nitrous oxide–anesthetized rats were subjected to ischemia by a high-pressure neck cuff and arterial hypotension for 0 (control), 3, 10, or 30 minutes (n=5 at each time). Brain temperature was allowed to fall spontaneously during ischemia, and the brain was frozen in situ with liquid N₂ without recirculation. The frontal cortex, hippocampus, and basal ganglia were dissected at —15°C. The lipids were separated by column and high-performance thin-layer chromatography and quantified by charring and densitometry.

Results: Total lipid content was higher (p<0.01) in the hippocampus (72.6±2.8 mg/g wet wt, mean±SD) than in the frontal cortex and basal ganglia (57.7±2.1 and 62.6±1.5 mg/g wet wt, respectively). Ischemic changes occurred only in the frontal cortex, where total lipid content fell (p<0.01) by 11% after 30 minutes of ischemia because sulfatide and cerebroside contents fell by 44% and 38%, respectively.

Conclusions: Despite a marked accumulation of free fatty acids during complete global brain ischemia in rats, the only detectable changes in brain lipids were in the amounts of cerebrosides and sulfatides in the frontal cortex.

KEY WORDS • cerebral ischemia • lipids • rats

Membrane lipid degradation is important in the pathogenesis of ischemic brain damage for several reasons. First, lipid degradation reflects breakdown of the integrity of neuronal membranes. Second, free fatty acids (FFAs) themselves, and especially polyunsaturated FFAs such as arachidonic acid, are precursors for other hormones such as prostaglandins and leukotrienes that have potent effects on cerebral perfusion and metabolism. Third, changes in membrane lipid composition can be expected to have profound effects on membrane function by affecting membrane-associated enzymes, receptors, and channels. Thus, a thorough understanding of membrane lipid degradation—which lipids are degraded, the mechanisms involved, and their impact on membrane function—is essential to understanding the mechanisms of ischemic brain damage.

Several groups of investigators have shown that the early and rapid increase in brain FFA content during ischemia is likely owing to the activation of phospholipases and the hydrolysis of phospholipids (PLs) in general and of polyphosphoinositides in particular. However, the changing patterns and rates of release of FFAs during ischemia and their responses to the effects of various calcium channel blockers suggest that either the origin, the mechanisms, or both, of FFA release are altered as ischemia is prolonged. Ischemic changes in brain PLs and FFAs have been extensively studied, but there is little information on changes in other lipid classes such as cerebrosides, sulfatides, and sphingomyelin (SPH) (which are found primarily in myelin) and in gangliosides (which are associated with neurons).

Reliable methods for quantifying these lipids in small amounts of tissue have recently been developed. Our objective was to provide a comprehensive analysis of regional changes in the major lipid classes during complete global brain ischemia. A model of complete brain ischemia eliminates the possibility that regional differences in ischemic brain lipid composition might be caused by differences in the severity of ischemia rather than differences in the metabolic response to ischemia.

Materials and Methods

In a protocol approved by the Institutional Animal Care and Use Committee, 20 male Wistar rats weighing 350–450 g were equally and randomly assigned to one of four study groups: nonischemic controls and rats subjected to 3, 10, or 30 minutes of ischemia. Anesthesia was induced with 4.0% halothane in oxygen, the trachea was intubated with a 14-gauge Intracath (Baxter Healthcare Corp., Deerfield, Ill.), and muscle paralysis
was maintained with 0.2 mg/hr i.v. pancuronium bromide (Elkins-Sinn, Inc., Cherry Hill, N.J.). Each rat was mechanically ventilated using a model 680 rodent respirator (Harvard Apparatus, South Natick, Mass.) with 0.5% halothane/70% N₂O/30% O₂. The femoral artery and vein were cannulated to monitor blood pressure (Grass Instrument Co., Quincy, Mass.), and 0.75 ml arterial blood was withdrawn for measurements of Paco₂, PaO₂, and arterial pH (model 176 trielectrode blood gas machine; Corning, Inc., Medfield, Mass.). Halothane was discontinued, and the rat was ventilated with 70% N₂O/30% O₂ for 30 minutes. Rectal temperature was continuously monitored and maintained at 38°C by a water heating pad (Gorman-Rupp Industries, Bellville, Ohio).

In three rats, cortical brain temperature was monitored with epidural thermistors during ischemia. Cortical temperature fell by as much as 5–7°C over 30 minutes. We chose to let brain temperature fall as it does during cardiac arrest in patients.

Complete global brain ischemia was produced by arterial hypotension and a high-pressure neck tourniquet as previously described.²² Briefly, mean arterial blood pressure (MABP) was lowered to 50 mm Hg by an intravenous injection of trimethaphan camsylate (Roche Laboratories, Nutley, N.J.), and the tourniquet was rapidly inflated to 1,500 mm Hg. After ischemia, the brains were frozen in situ with liquid nitrogen.²³ During freezing, MABP was maintained at 80–100 mm Hg by an intravenous injection of 0.01 mg/ml norepinephrine (Breon Labs, New York, N.Y.). Control rats’ brains were frozen in situ after 30 minutes of postsurgical stabilization.

The brain regions were dissected at -15°C. Thirty- to 50-mg samples of the frontal cortex, hippocampus, and basal ganglia were placed in preweighed test tubes containing 2.5 ml of a chloroform/methanol mixture (1:1 by volume). The tissue was homogenized for 3 minutes (Polytron homogenizer, Brinkmann Instruments, Inc., Westbury, N.Y.) and centrifuged at 1,000g (model JB-6 centrifuge, Beckman Instruments, Inc., Waldwick, N.J.) for 10 minutes. The samples were then treated as previously described by Macala et al.²⁰ Briefly, neutral and acidic lipids were separated on Bond-Elute NH₄ columns (Analytichem Int., Harbor City, Calif.) and subjected to high-performance thin-layer chromatography (HPTLC).

Lipid standards in chloroform/methanol (2:1 by volume) were obtained from Sigma Chemical Co., St. Louis, Mo. All solvents used for chromatography were of high-performance liquid chromatography purity (Fisher Scientific, Malvern, Pa.). HPTLC plates (Silica Gel 60, 10x20 cm, 0.20 mm, Merck, Darmstadt, FRG) were prewashed with chloroform/methanol/water (60:35:8 by volume), activated at 110°C for 15 minutes, and then cooled in a vacuum desiccator. Five microlitters of the brain lipid samples and lipid standards were quantified by HPTLC.

The lipids were quantified by reflectance densitometry²⁴ (model 620 video densitometer, Bio-Rad, Richmond, Calif.) using 1-D ANALYST software.

Groups were compared using one-way analysis of variance and Dunnett’s test, with p<0.05 the level of significance.

### Results

Physiological variables measured before (control) and after ischemia of varying durations were similar (Table 1).

Total lipid content in the frontal cortex (Table 2) was not significantly altered after 3 or 10 minutes of ischemia; after 30 minutes, however, total lipid content decreased by 11% (p<0.01). Cerebroside content in the frontal cortex decreased progressively with increasing durations of ischemia and after 30 minutes was significantly (p<0.01) lower than control values. Sulfatide content fell progressively, and values were lower than control after all durations of ischemia. Total lipid content in the hippocampus of the controls was 72.6±2.8 mg/g wet wt, higher (p<0.01) than in the frontal cortex. However, contents of neither total nor individual lipid fractions in the hippocampus were altered during up to 30 minutes of ischemia (Table 3).

In the basal ganglia (Table 4), total lipid content was significantly lower (p<0.01) than in the hippocampus. As in the hippocampus, however, in the basal ganglia neither total nor individual lipid fractions were altered by ischemia.

### Table 1. Physiological Variables in Rats Before Complete Global Brain Ischemia by Arterial Hypotension and High-Pressure Neck Tourniquet

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean arterial blood pressure (mm Hg)</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>pHa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>132±8</td>
<td>146±24</td>
<td>37±2</td>
<td>7.38±0.04</td>
</tr>
<tr>
<td>Ischemia</td>
<td>132±8</td>
<td>146±24</td>
<td>37±2</td>
<td>7.38±0.04</td>
</tr>
</tbody>
</table>

*Values are mean±SD; n=5 for each group.

### Table 2. Lipid Composition in Frontal Cortex of Rats Subjected to Complete Global Brain Ischemia by Arterial Hypotension and High-Pressure Neck Tourniquet

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipids</th>
<th>Sphingomyelin</th>
<th>Phosphatidylycerine</th>
<th>Phosphatidylethanolamine</th>
<th>Cerebroside</th>
<th>Cholesterol</th>
<th>Phosphatidylserine</th>
<th>Phosphatidylserine</th>
<th>Sulfatides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.7±2.1</td>
<td>2.7±0.3</td>
<td>27.9±2.6</td>
<td>25.8±1.4</td>
<td>8.3±1.2</td>
<td>21.6±1.7</td>
<td>2.1±0.4</td>
<td>8.0±1.1</td>
<td>3.6±1.1</td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min</td>
<td>59.2±2.0</td>
<td>2.6±0.4</td>
<td>30.2±1.3</td>
<td>26.8±1.3</td>
<td>6.8±1.8</td>
<td>22.1±1.2</td>
<td>2.0±0.1</td>
<td>7.6±1.0</td>
<td>1.8±0.4*</td>
</tr>
<tr>
<td>10 min</td>
<td>54.6±0.7</td>
<td>2.4±0.4</td>
<td>27.7±3.8</td>
<td>27.7±1.2</td>
<td>6.6±2.2</td>
<td>23.3±1.4</td>
<td>2.4±0.5</td>
<td>8.3±1.7</td>
<td>1.6±0.7*</td>
</tr>
<tr>
<td>30 min</td>
<td>51.5±2.6</td>
<td>2.5±0.3</td>
<td>31.2±1.0</td>
<td>27.7±1.5</td>
<td>5.2±1.0t</td>
<td>21.6±2.3</td>
<td>2.1±0.3</td>
<td>7.7±1.0</td>
<td>2.0±0.5*</td>
</tr>
</tbody>
</table>

*Values are mean±SD mg/g wet wt for total lipids and mean±SD % of total lipids for individual lipid classes; n=5 for each group.

*p<0.05, p<0.01 different from controls.
TABLE 3. Lipid Composition in Hippocampus of Rat Brains Subjected to Complete Global Brain Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipids</th>
<th>Sphingomyelin</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylethanolamine</th>
<th>Cerebrosides</th>
<th>Cholesterol</th>
<th>Phosphatidylinositol</th>
<th>Phosphatidylserine</th>
<th>Sulfatides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>72.6±2.8</td>
<td>2.7±0.6</td>
<td>24.4±1.4</td>
<td>23.5±1.0</td>
<td>13.1±2.6</td>
<td>23.1±1.7</td>
<td>13.0±2.0</td>
<td>7.4±1.1</td>
<td>4.4±0.8</td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min</td>
<td>71.5±2.9</td>
<td>2.8±0.4</td>
<td>26.0±1.5</td>
<td>23.2±0.6</td>
<td>11.3±0.7</td>
<td>23.3±1.8</td>
<td>14.0±2.0</td>
<td>7.9±0.6</td>
<td>4.2±0.4</td>
</tr>
<tr>
<td>10 min</td>
<td>74.0±9.3</td>
<td>2.8±0.4</td>
<td>25.1±0.9</td>
<td>23.3±1.0</td>
<td>12.3±1.6</td>
<td>23.7±1.2</td>
<td>15.0±3.0</td>
<td>7.6±0.8</td>
<td>4.0±1.0</td>
</tr>
<tr>
<td>30 min</td>
<td>69.8±3.2</td>
<td>2.7±0.2</td>
<td>25.5±1.7</td>
<td>23.9±1.4</td>
<td>11.5±2.1</td>
<td>23.7±1.5</td>
<td>16.0±3.0</td>
<td>7.8±0.9</td>
<td>3.3±1.5</td>
</tr>
</tbody>
</table>

Values are mean±SD mg/g wet wt for total lipids and mean±SD % of total lipids for individual lipid classes; n=5 for each group.

In all brain regions, hydroxy fatty acid (HFA) cerebrosides contributed 60-70% of the total cerebrosides, whereas nonhydroxy fatty acid (NFA) cerebrosides contributed the remaining 30-40% (Table 5). Concentrations of both isomer forms decreased after 30 minutes of ischemia in the frontal cortex; however, the decrease in the NFA form (55%) was greater than that in the HFA form (40%).

Discussion

The precautionary measures of in situ freezing and dissection at −15°C were taken to avoid postmortem changes in brain lipids. Brain lipids quantified 48 hours post mortem reportedly compare favorably with those from biopsy specimens obtained intraoperatively. However, the rapid accumulation of brain FFAs during ischemia clearly shows that lipid hydrolysis occurs rapidly during ischemia. Furthermore, although total lipid levels in the frontal cortex, basal ganglia, and hippocampus were similar to the levels in human brain reported by Soderberg et al, our data and those of Norton and Poduslo show higher PL levels, which may be owing to the 24-hour postmortem delay before analysis of the samples of Soderberg et al.

The relative lack of change in brain lipids during complete global brain ischemia is striking. Aside from the decrease in the cerebrosides content after 30 minutes of ischemia and the progressive fall in the sulfatide content, amounts of other lipids such as PL were remarkably unchanged. This may be consistent with the absence of histological changes observed during complete global brain ischemia as reported by Kalimo et al.

Brain PL levels during ischemia have been extensively studied, but the results vary. DeMedio et al reported that 10 minutes of ischemia decreased the total PL content by 8%, the SPH content by 10%, the phosphatidylcholine (PC) content by 15%, the phosphatidylethanolamine (PE) content by 4%, and the phosphatidylinositol (PI) content by 23% in gerbils. Yoshida et al reported that after 30 minutes of ischemia, the PE content decreased by 16%. Enseleit et al reported that no change occurred in the total PL content, while the SPH content decreased by 50% after 15 minutes and the PE, phosphatidylserine, PI, and phosphatic acid contents decreased significantly after 4 hours of ischemia. Partial ischemia or hypoperfusion appear to cause greater changes in brain lipids and histopathology than complete global brain ischemia.

Rehncrona et al and Hattori et al reported that 30 minutes of complete or incomplete ischemia did not cause any change in total or individual PL contents in rats. In contrast, Goto et al showed that the PE content decreased after 5 minutes of ischemia, with no change in the PC content after 60 minutes. These results suggest that in models of complete global brain ischemia, the PL content changes little if any. In contrast, consistent clear-cut decreases in the amount of polyphosphatidylinositol occur during ischemia with a reduction in the PI content, suggesting a differential substrate preference of phospholipase C.

Our results show differences in the regional responses to ischemia. Of the brain regions examined, only the frontal cortex was affected and only with respect to cerebrosides and sulfatides. Bhakoo et al showed no significant difference in FFA accumulations among the frontal, parietal, and occipital cortices after 1 hour of bilateral carotid occlusion in gerbils; the PL contents were also unaltered. However, arachidonic acid accumulation was greater in the more vulnerable CA1 region than in the less vulnerable CA3 region of rat hippocampus after 8 and 12 minutes of global ischemia, indicating that regional differences at the level of FFA accumulation can be detected.

Regional sulfatide levels in our data show a greater effect in the frontal cortex than in the basal ganglia or hippocampus, which contrasts with results obtained in the four-vessel occlusion model in which the hippocampus shows the earliest histological damage. The lack of similar changes in the hippocampus and basal ganglia

TABLE 4. Lipid Composition in Basal Ganglia of Rats Subjected to Complete Global Brain Ischemia by Arterial Hypotension and High-Pressure Neck Tourniquet

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lipids</th>
<th>Sphingomyelin</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylethanolamine</th>
<th>Cerebrosides</th>
<th>Cholesterol</th>
<th>Phosphatidylinositol</th>
<th>Phosphatidylserine</th>
<th>Sulfatides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.6±1.5</td>
<td>3.1±0.5</td>
<td>27.0±1.4</td>
<td>23.7±0.9</td>
<td>11.5±1.0</td>
<td>21.6±1.2</td>
<td>1.7±0.1</td>
<td>7.6±0.5</td>
<td>3.8±0.8</td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min</td>
<td>65.4±2.5</td>
<td>3.3±0.5</td>
<td>28.1±2.0</td>
<td>24.6±0.7</td>
<td>9.7±0.9</td>
<td>21.4±1.6</td>
<td>1.7±0.3</td>
<td>8.2±0.7</td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>10 min</td>
<td>66.6±2.9</td>
<td>3.3±0.5</td>
<td>27.6±1.8</td>
<td>25.4±1.0</td>
<td>12.2±1.4</td>
<td>19.9±1.5</td>
<td>1.5±0.2</td>
<td>7.3±1.0</td>
<td>2.8±1.0</td>
</tr>
<tr>
<td>30 min</td>
<td>65.8±1.2</td>
<td>3.2±0.3</td>
<td>27.6±1.3</td>
<td>24.2±1.3</td>
<td>11.1±1.4</td>
<td>20.4±2.3</td>
<td>1.9±0.4</td>
<td>7.5±1.4</td>
<td>4.1±0.9</td>
</tr>
</tbody>
</table>

Values are mean±SD mg/g wet wt for total lipids and mean±SD % of total lipids for individual lipid classes; n=5 for each group.
may be explained by their different lipid compositions compared with the frontal cortex. The higher lipid and cerebroside contents of the hippocampus compared with the frontal cortex correlates with the higher myelin content of nervous tissue.26,39,40 Histological analysis of the brain after ischemia indicates that myelin is relatively resistant to ischemic damage and that disruption of the myelin structure occurs during the final period of ischemic tissue injury.41 Why the cerebrosides and sulfatides are affected to a greater extent in the frontal cortex than in other brain regions is unknown. It should be noted, however, that changes in other lipid fractions were not detectable at this level of analysis but may be found with, for example, not only FFA analysis but different PL molecular species analysis.

Decreases in the levels of cerebrosides and sulfatides after ischemia have not been previously reported. Ensell et al.41 reported a 50% decrease in the cerebral content of SPH after 15 minutes of ischemia. Galactocerebrosides and sulfatides are major constituent lipids of the myelin sheath, and their distributions in the brain and maturational changes coincide with the myelination process. As much as 94% of the cerebroside content and 95% of the sulfatide content of the total white matter were recovered in large and small myelin fractions, whereas all other subcellular fractions contained only small amounts of these lipids. The distribution of sphingolipids was similar in both gray and white matter. However, the sphingolipid content in gray matter was much lower, and the isomer composition of cerebrosides in gray matter synaptosomal, mitochondrial, and microsomal fractions differed from that in the white matter nonmyelin pool.42

Cerebrosides and sulfatides are hydrolyzed by enzymes of lysosomal origin.43 Their degradation under physiological conditions requires activator proteins and an acidic pH. Ischemic injury results in a marked shift of hydrolyses to the soluble phase in the liver44 and myocardium.45 However, the importance of lysosomal hydrolyses in ischemic brain damage has not been established. Their release into the cytoplasm occurs later during ischemic injury, along with profound neuropathologic and functional damage to nervous tissue.46,47 Cerebrosides and sulfatides have a slow metabolic turnover in normal adult brain,48 so the decreases in their concentrations during ischemia could be caused by a decrease in intracellular pH.

Detailed studies of cerebroside and sulfatide compositions in gray and white matter revealed different isomer compositions in neurons and myelin.42 Despite a much lower cerebroside content, neurons contain hydrolyzing enzymes at levels of activity similar to those in myelin.49 The presence of a high level of hydrolyzing activity and the lack of binding proteins to deactivate the enzymes render neuronal cerebrosides and sulfatides susceptible to attack by hydrolases, especially when pH or ionic strength in the cell changes. Two metabolic pools of cerebrosides with different turnover rates were identified after the injection of a labeled precursor into brain,40 consistent with the notion that the rapidly metabolizing cerebroside could be in gray matter and the slowly metabolizing fraction could be myelin-bound. The relatively higher proportion of HFA cerebrosides compared with NFA cerebrosides in the frontal cortex corroborates previous data published by others.42 Cerebrosides in enriched neuronal and glial fractions from rat brain contain predominantly C18-C20 NFA and very little C16-C20 NFA or HFA, whereas in myelin these relative quantities are reversed.51 The decrease in cerebroside levels in the frontal cortex after 30 minutes of ischemia involves both cerebroside isomers; however, there is a relatively greater degradation of the NFA portion. The difference is probably due to the higher content of 18:0 in NFA cerebrosides and to its lower lipid content, which make it more susceptible to degradation by hydrolase.52

In summary, except for decreases in the amounts of cerebrosides and sulfatides, no significant changes in individual lipid fractions were observed during complete global brain ischemia in rats. Studies on the changes occurring with recirculation are now in progress.

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