Lipid Metabolism, Cerebral Metabolic Rate, and Some Related Enzyme Activities After Brain Infarction in Rats

Jean Bralet, Paulette Beley, Riadh Jemaa, Anne-Marie Bralet, and Alain Beley

Multiple infarcts were produced in cerebral hemispheres of rats by injecting calibrated 50-μm microspheres into the left internal carotid artery, and alterations in lipid and energy metabolism were evaluated 24 hours later in the embolized hemisphere. Total phospholipid content was decreased by 26%, but the different classes of phospholipids were not equally affected. Phosphatidylinositol and phosphatidylserine levels were decreased by about 40% and phosphatidylcholine and phosphatidylethanolamine by 25%, while sphingomyelin level remained unchanged. There was a 3.2-fold increase in total free fatty acid content with a relatively larger rise in polyunsaturated free fatty acids 20:4 and 22:6 (20-fold increase). Determination of enzyme activities showed decreases in Na⁺,K⁺-ATPase (-21%) and hexokinase (-14%) but no changes in phosphofructokinase and pyruvate kinase. Study of energy metabolism using the closed system method of Lowry et al showed a significant depression (-36%) of the cerebral metabolic rate. Taken together, these data suggest a relation between lipid alterations and dysfunction of energy metabolism. Phospholipid degradation with subsequent free fatty acid release and alteration in membrane-bound enzymes may have a direct effect on metabolic machinery and may slow cerebral metabolic rate. (Stroke 1987; 18:418-425)

ISCHEMIA initiates a progression of biochemical events that can lead to irreversible damage and cell death. The metabolic changes that occur after induction of a generalized brain ischemia and the recovery following a period of transient ischemia have been extensively studied.1 In focal and multifocal ischemia, the situation is more complex, resulting from the juxtaposition of ischemic and nonischemic regions. Alterations and disruption of cells within the ischemic focus may release a wide variety of compounds causing vascular and metabolic effects in the surrounding regions. Among the biochemical events occurring during ischemia, membrane alterations may have important consequences on the metabolism and function of the brain. Membranes and membrane-bound enzymes, especially Na⁺,K⁺-ATPase, play a crucial role in energy metabolism, the transport of Na⁺ and K⁺ ions accounting for about half of the brain energy consumption.2 Free fatty acids (FFA), which are liberated from membrane phospholipids (PL) during ischemia,3 are known to impair mitochondrial function4 and to inhibit the Na⁺,K⁺-ATPase activity.5-8 Arachidonic acid metabolites, prostaglandins, may induce alterations in the blood–brain barrier9 and disturbances of neuronal function.10 All these data suggest a link between lipid alterations and dysfunction of energy metabolism. However, most data about the effects of ischemia on PL, FFA, or energy metabolism have been obtained separately using various experimental models. The purpose of this study was to observe the simultaneous changes in PL, FFA, and energy metabolism that occur 24 hours after induction of brain infarction. Multifocal infarction was induced in rat cerebral hemispheres by intracarotid injection of calibrated microspheres,11 and the metabolic state of the embolized hemisphere was evaluated by quantifying PL and FFA, by measuring the cerebral metabolic rate according to the method of Lowry et al,12 and by determining the activity of some enzymes implicated in energy metabolism.

Materials and Methods

The experiments were performed on male Sprague-Dawley rats weighing 280–320 g, fed ad libitum.

Production and Evaluation of Embolization

Cerebral microembolism was produced by injecting 4,000 carbonized microspheres (50 μm diameter, labelled with strontium-85, suspended in 20% polyvinylpyrrolidone) into the left internal carotid artery as previously described.11 The mean radioactivity present in 1 microsphere was determined by simultaneous counting of the microsphere suspension in a hemocytometer and a scintillation crystal well counter to calculate the number of microspheres contained in the cerebral hemisphere.

Histology

Twenty-four hours after embolization, the rats (n = 10) were anesthetized (chloral hydrate, 360 mg/kg, i.p.), heparinized (1,000 IU, i.v.) and the brains were fixed by in vivo perfusion.13 The chest was opened, the right atrium was incised, the abdominal
Bralet et al Lipid and Energy Metabolism in Brain Infarction

419

aorta was clamped, and physiological saline from a reservoir 110 cm above heart level was allowed to perfuse the cephalic circulation for 30 seconds. This was followed by perfusion with about 100 ml of FAM (40% formaldehyde:acetic acid:methanol, 1:1:8 by vol.). The brains were removed 4 hours later and stored in FAM until they were embedded in paraffin. Coronal sections (10 μm) were stained with Luxol fast blue and cresyl violet and examined with the light microscope.

Energy Metabolism

Control and 24-hour embolized rats were initially anesthetized by inhalation of ether and tracheotomized. Polyethylene catheters were inserted in the femoral arteries for continuous blood pressure recording and anaerobic blood sampling. Thereafter the animals were paralyzed with tubocurarine chloride (1 mg/kg, i.v.) and connected to a respirator that delivered 70% N2O and 30% O2. Arterial P, Pco2, and pH were measured using direct-reading electrodes. Body temperature was kept close to 37° C by external heating. A skin incision was made to fit a plastic funnel over the skull for freezing the brain in situ by pouring liquid nitrogen into the plastic funnel during 3 minutes. Thereafter the animal was submersed in liquid nitrogen for another 5 minutes. In another series of experiments, control and 24-hour embolized rats were decapitated and the heads immediately immersed in liquid nitrogen without stirring for 8 minutes.

The frozen brains were chiselled out of the skull, and the left hemispheres were isolated. Deep structures were discarded during irrigation with liquid nitrogen, and only the supratentorial cortical tissue, about 3 mm in dimension followed by chloroform:acetone:methanol:ammonia (d = 0.92):water (55:25:43:1:7 by vol.) in the first dimension followed by chloroform:acetone:methanol:acetic acid:water (50:20:10:10:4.7 by vol.) in the second dimension. PL were separated into 5 classes (phosphatidylcholine, PC; phosphatidylethanolamine, PE; phosphatidylerine, PS; phosphatidylinositol, PI; sphingomyelin, SM) by two-dimensional thin layer chromatography using tetraphydrofurane:acetone:methanol:ammonia (90:20:2:3 by vol.) and determined with pentadecanoic acid as an internal standard. The following fatty acids were quantified: palmitic (16:0), stearic (18:0), oleic (18:1), arachidonic (20:4), and docosahexaenoic (22:6). The values were expressed as nmol/g wet wt.

Lipid Analysis

The brains of control and 24-hour embolized rats were removed after freezing in situ, and the left hemispheres were isolated as described. Total lipids were extracted by homogenizing the hemisphere in chloroform:methanol (1:1 by vol.) using an Ultra-Turrax homogenizer. The homogenate was filtered, and the residue was subjected to scintillation counting for determination of the microspheres. Neutral lipids were separated from polar lipids by column chromatography. FFA were isolated from the neutral lipid frac-

tion by one-dimensional thin layer chromatography on silica gel using n-hexane:diethylether:acetic acid:methanol (90:20:2:3 by vol.) and determined with gas–liquid chromatography using pentadecanoic acid as an internal standard. The values were expressed as nmol/g wet wt.

Enzyme Assay

Control and 24-hour embolized rats were decapitated, the left cerebral cortex was rapidly removed into 20 vol. of ice-cold isolation medium (Tris-HCl buffer 5 mM, pH 7.4), and homogenized using a motor-driven Teflon-glass homogenizer (10 strokes, 750 rpm). The radioactivity present in the cortex homogenate and in the remainder of the hemisphere was measured to calculate the number of microspheres present in the hemisphere. All the assays were carried out at 25° C on samples from cortex homogenates in toto. Protein content was determined according to Lowry et al. The maximal rate of the following enzymatic activities was evaluated by graphic recording using nicotinamide adenine dinucleotide (NADP)-dependent systems and expressed as nmol substrate/min/mg protein: hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11), pyruvate kinase (EC 2.7.1.40). Na+,K+-ATPase (EC 3.6.1.3) activity was measured by determining the amount of phosphate released by enzymatic cleavage of ATP in absence or presence of ouabain according to MacMillan. Pi was determined by the phosphomolybdate method and results expressed as nmol Pi produced/min/mg protein.

Calculations

The means ± SEM were determined, and statistical evaluations were performed with the two-tailed Student's t test.

Metabolic rate. Metabolic rate was measured by the change in energy-rich metabolites using the method of Lowry et al., which is based on the concept that during a brief period of ischemia the changes in P Cr, ATP, ADP, glucose, and glycogen reflect the in vivo rate of energy utilization. The changes in each metabolite and in the energy reserve were calculated from the differences between mean values in brains frozen in situ with an intact circulation and those frozen after decapitation. Since glycogen was not used during the
short period of ischemia following decapitation (see below), the potential energy reserve \( (-P \mu mol/g) \) utilization was estimated from the formula

\[
\Delta \sim P = \Delta PCr + 2 \Delta ATP + \Delta ADP + 2 \Delta glucose
\]

which should give a valid estimate of \( -P \) flux. The errors in the differences were calculated in the following way:\(^2\)

\[
(A \pm a) - (B \pm b) = (A - B) \pm \sqrt{a^2 + b^2}
\]

where \( (A \pm a) \) = mean value obtained in brains frozen in situ, affected by its SEM, \( (B \pm b) \) = mean value obtained in brains after decapitation and freezing of the heads, affected by its SEM, \( (A - B) \pm \sqrt{a^2 + b^2} \) = metabolite change, affected by its SEM.

**Results**

**Control of Embolization**

The degree of embolization was determined in each experiment by calculating the number of microspheres present in the embolized hemisphere. The latter was found equal to 486 ± 61, 480 ± 70, and 442 ± 53, respectively, in animals used for the determination of energy metabolism, PL and FFA, and enzyme activities. There were no significant differences between the experimental groups.

**Histology**

Examination of brains 24 hours after microsphere injection (Figure 1) showed swelling of the left embolized hemisphere secondary to the development of edema. The microspheres were located in both deep and cortical circulation, and infarcts of variable size were located in the left hemisphere, characterized by well-demarcated areas of necrosis in which the tissue was poorly stained. There was a loss of neurons and, to a lesser extent, of astrocytes within the infarcted regions. The remaining cells were shrunken and showed a pyknotic and darkly staining nucleus, but at this time there was no macrophage or glial proliferation. Although the distribution of the infarcts was unpredictable, the structures most frequently involved were the parieto-temporal cortex, the thalamostriate areas, and the hippocampus.

**Energy Metabolism**

The physiological parameters of the animals at the time of freezing of the brain in situ showed no significant differences between control \( (n = 8) \) and embolized rats \( (n = 8) \). Respectively, the values were 7.37 ± 0.01 and 7.38 ± 0.01 for arterial pH, 38.7 ± 0.7 and 38.2 ± 0.8 mm Hg for \( P_{aco_2} \), 138 ± 7 and 144 ± 6 mm Hg for \( P_{ao_2} \), 117 ± 5 and 116 ± 6 mm Hg for mean arterial blood pressure, and 36.9 ± 0.1 and 36.8 ± 0.1°C for body temperature.

Embolization caused marked changes in energy metabolite levels (Table 1), consisting of decreases in PCr \((-28\%)\), ATP \((-29\%)\), and ADP \((-23\%)\) and increases in lactate \((+213\%)\) and glycogen \((+254\%)\). There was a loss of adenine nucleotides \((-28\%)\), and the potential energy reserve was reduced by 16%.

Freezing the rat head in liquid nitrogen immediately after decapitation resulted in marked metabolic changes (Table 1), due to the time lag necessary for freezing of the cerebral cortex. In control rats, there were decreases in PCr \((-78\%)\), ATP \((-24\%)\), and glucose \((-94\%)\) and concomitant increases in ADP, AMP, and lactate. The changes were less marked in embolized rats (PCr, -62%; ATP, -19%; glucose, -63%). Calculation of the metabolite changes in response to decapitation showed differences between control and embolized rats (Table 2). Embolized cortex showed diminutions in the utilization of PCr \((2.13 \text{ vs. } 3.73 \mu mol/g \text{ in control, } p<0.01)\), glucose \((2.92 \text{ vs. } 4.40 \mu mol/g \text{ in control, } p<0.05)\), ATP \((0.40 \text{ vs. } 0.73 \mu mol/g \text{ in control, not significant})\) and reductions in the production of ADP \((0.057 \text{ vs. } 0.341 \mu mol/g \text{ in control, } p<0.001)\) and AMP \((0.147 \mu mol/g \text{ in control, not significant})\). The utilization of the potential energy reserve was significantly reduced in the embolized group \((p<0.01)\), reaching only 64% of the control value.

Glycogen was not significantly used in either control or embolized rats, so it was not incorporated into the energy use equation. Its incorporation into the calculation proposed by Lowry et al\(^{12}\) (glycogen level × 2.9) attenuated the difference between control and embolized animals, the energy use in embolized rats being reduced by 27% instead of 36%. In both groups the glucose disappearance in response to decapitation was found to exceed the theoretical production of lactate \((\text{glucose} \times 2)\), presumably owing to the accumulation of glycolysis intermediates.

**Phospholipids**

Table 3 gives the total and individual PL content in cerebral hemispheres of control and embolized rats. Embolization led to a significant fall in total PL \((-26\%)\), but the different classes were not equally reduced. PI level was reduced by 40%, PS by 37%, PC
and PE by 25%, whereas SM was not significantly modified.

**Free Fatty Acids**

The main FFA content in control and embolized hemispheres is reported in Table 4. In control rats, the level of total FFA reached 169 nmol/g, 16:0, 18:0, and 18:1 being the most prominent components. A striking rise in all the FFA was seen in the embolized rats, total FFA concentration reaching 541 nmol/g (3.2-fold increase). There was a relatively larger rise in the polyenoic acids 20:4 and 22:6 (20-fold increase) as compared to the other FFA (2.3- to 3-fold increase).

**Enzyme Activities**

As shown in Table 5, the pyruvate kinase and phosphofructokinase activities in cortex homogenates were not significantly different in control and embolized rats, whereas significant decreases in Na⁺,K⁺-ATPase (−21%) and hexokinase (−14%) activities were found after embolization.

**Discussion**

Cerebral microembolization with calibrated microspheres is a relatively simple and convenient method for studies of various aspects of cerebral infarction in small experimental animals. The use of radioactive microspheres as embolic agents enabled the number of microspheres to be determined and allowed comparisons between the different series of experiments. Some characteristics of this stroke model have been previously reported.11,30,31 Twenty-four hours after microsphere injection, the time of the present study, regional cerebral blood flow in the embolized hemisphere reached 30–50% of the control value, depending on the cerebral structure.

**Lipid Metabolism**

The effect of ischemia on cerebral PL content at short times after induction of brain ischemia has been reported. In rats, Rehncrona et al32 showed that neither complete nor incomplete ischemia of 30-minute dura-

### Table 1. Effect of Embolization on Energy Metabolite Levels in Cerebral Cortex Frozen In Situ (with an Intact Circulation) or Immediately After Decapitation

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (n = 8)</th>
<th>Embolized (n = 8)</th>
<th>Control (n = 8)</th>
<th>Embolized (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr</td>
<td>4.75 ± 0.15</td>
<td>3.42 ± 0.48*</td>
<td>1.02 ± 0.14</td>
<td>1.29 ± 0.20</td>
</tr>
<tr>
<td>ATP</td>
<td>3.03 ± 0.14</td>
<td>2.14 ± 0.26†</td>
<td>2.30 ± 0.12</td>
<td>1.74 ± 0.23</td>
</tr>
<tr>
<td>ADP</td>
<td>0.269 ± 0.012</td>
<td>0.208 ± 0.012†</td>
<td>0.610 ± 0.054</td>
<td>0.265 ± 0.046</td>
</tr>
<tr>
<td>AMP</td>
<td>0.029 ± 0.003</td>
<td>0.037 ± 0.003</td>
<td>0.176 ± 0.037</td>
<td>0.105 ± 0.017</td>
</tr>
<tr>
<td>Sum of nucleotides</td>
<td>3.33 ± 0.14</td>
<td>2.38 ± 0.27†</td>
<td>3.09 ± 0.10</td>
<td>2.11 ± 0.26</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.70 ± 0.45</td>
<td>4.61 ± 0.45</td>
<td>0.30 ± 0.05</td>
<td>1.69 ± 0.33</td>
</tr>
<tr>
<td>Glycogen</td>
<td>1.40 ± 0.13</td>
<td>4.96 ± 0.80†</td>
<td>1.77 ± 0.16</td>
<td>4.75 ± 1.01</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.92 ± 0.18</td>
<td>9.13 ± 1.35‡</td>
<td>4.91 ± 0.38</td>
<td>10.92 ± 2.37</td>
</tr>
<tr>
<td>Potential energy reserve</td>
<td>20.48 ± 0.89</td>
<td>17.15 ± 1.13*</td>
<td>6.88 ± 0.23</td>
<td>8.42 ± 0.82</td>
</tr>
</tbody>
</table>

Embolized rats were sacrificed 24 hours after injection of microspheres into the left internal carotid artery, and metabolites were measured in the left embolized cortex. Values are means ± SEM; n = number of animals; sum of nucleotides = ATP + ADP + AMP; potential energy reserve = PCr + 2 ATP + ADP + 2 glucose.

*p < 0.05, †p < 0.01, ‡p < 0.001 between control and embolized rats after freezing of the brain in situ.

### Table 2. Effect of Embolization on Cerebral Metabolic Rate

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>Embolized</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr</td>
<td>−3.73 ± 0.20</td>
<td>−2.13 ± 0.52*</td>
</tr>
<tr>
<td>ATP</td>
<td>−0.73 ± 0.18</td>
<td>−0.40 ± 0.34</td>
</tr>
<tr>
<td>ADP</td>
<td>0.341 ± 0.055</td>
<td>0.057 ± 0.047†</td>
</tr>
<tr>
<td>AMP</td>
<td>0.147 ± 0.037</td>
<td>0.068 ± 0.017</td>
</tr>
<tr>
<td>Glucose</td>
<td>−4.40 ± 0.45</td>
<td>−2.92 ± 0.55‡</td>
</tr>
<tr>
<td>Glycogen</td>
<td>0.37 ± 0.20</td>
<td>−0.21 ± 1.29</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.99 ± 0.42</td>
<td>1.79 ± 2.72</td>
</tr>
<tr>
<td>Potential energy reserve</td>
<td>−13.60 ± 0.92</td>
<td>−8.73 ± 1.39*</td>
</tr>
</tbody>
</table>

Change was the difference between the mean values in cerebral cortex frozen in situ and cerebral cortex frozen after decapitation (data of Table 1). − indicates decrease of the metabolite level; values are means ± SEM; potential energy reserve = PCr + 2 ATP + ADP + 2 glucose.

*p < 0.01, †p < 0.001, ‡p < 0.05 between control and embolized rats.

### Table 3. Effect of Embolization on Cerebral Phospholipids

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Control (n = 5)</th>
<th>Embolized (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine (PC)</td>
<td>22.52 ± 0.63</td>
<td>16.83 ± 0.99*</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (PE)</td>
<td>20.36 ± 0.88</td>
<td>15.15 ± 1.21*</td>
</tr>
<tr>
<td>Phosphatidylserine (PS)</td>
<td>5.54 ± 0.48</td>
<td>3.52 ± 0.43†</td>
</tr>
<tr>
<td>Phosphatidylinositol (PI)</td>
<td>1.44 ± 0.15</td>
<td>0.87 ± 0.12†</td>
</tr>
<tr>
<td>Sphingomyelin (SM)</td>
<td>2.90 ± 0.13</td>
<td>2.51 ± 0.29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>54.34 ± 1.63</td>
<td>40.21 ± 2.98*</td>
</tr>
</tbody>
</table>

Embolized rats were sacrificed 24 hours after injection of microspheres into the left internal carotid artery, and phospholipids were measured in the left embolized hemisphere. Values are means ± SEM; n = number of animals.

*p < 0.01, †p < 0.05 between control and embolized rats.
tion caused any measurable changes in levels of total or individual PL. In gerbils submitted to bilateral carotid artery occlusion, Enseleit et al. 33 indicated that total PL content was not significantly altered after 4 hours of occlusion. However, selective decreases in the levels of individual PL have been occasionally reported after short periods of ischemia. 33-35 Our results showing that prolonged ischemia induces a significant fall (-26%) in total PL content denote probably irreversible cell damage in infarcted brain regions. Nevertheless, the different classes of PL were not equally affected. PI and PS levels were reduced by about 40%, while PE and PC decreased by only 25%, and SM remained unaltered. A reduction in PL level reflects an imbalance between energy-dependent synthesis and breakdown, and the two processes can be implicated in the PL loss. The inhomogeneous reduction of the various classes may be influenced by differences in their turnover. In this connection, among the PL, PI has the fastest and SM the slowest turnover. 36

Increases in cerebral FFA levels, especially arachidonic acid, have been shown to occur at short times after induction of complete and incomplete brain ischemia. 33-35 Twenty-four hours after embolization, we observed a 3.2-fold increase in total FFA and a 20-fold increase in arachidonic and docosahexaenoic acid level. This increase is relatively moderate in comparison with those that have been reported after severe, generalized brain ischemia. The size of the FFA pool increased about 10 times after a 30-minute period of ischemia induced in gerbils by bilateral carotid artery occlusion 34 or in rats by carotid clamping associated with hypotension. 35 Considering the heterogeneity of our ischemic model, the FFA levels in the whole hemisphere reflect an averaging of normal and damaged tissue, and it is to be expected that higher concentrations of FFA are present within or around the infarcted areas. The release of FFA, especially polyunsaturated FFA, from membranes of damaged cells and their diffusion toward the adjacent areas can lead to mitochondrial dysfunction 4 and alteration in energy metabolism by inhibiting Na⁺,K⁺-ATPase. 5,4 Chan et al. 41 have shown that intracerebral injection of arachidonic acid at concentrations of 1-5 mM/l induced edema and decreased Na⁺,K⁺-ATPase activity. After bilateral carotid artery occlusion in rats for 6 hours, Kuwashima et al. 32 observed a continuous increase in FFA level with a progressive deterioration of cerebral energy metabolism, suggesting a relation between the two processes.

### Enzyme Activities

Among the tested enzymes, only the membrane-bound enzymes, Na⁺,K⁺-ATPase and hexokinase, showed significant reduction in their activities. The reported effects of ischemia on Na⁺,K⁺-ATPase activity are not consistent and seem to depend on the ischemic model. In gerbils submitted to carotid artery occlusion, enzyme activity was reduced after 15 39 or 30 minutes 33 and unaltered by up to 6 hours of ischemia. 40 In rats, enzyme activity increased during a 30-minute ischemic exposure induced by 4-vessel occlusion 27 and decreased after 5 minutes of complete ischemia induced by decapitation. 41 Decreases in Na⁺,K⁺-ATPase have been reported after induction of cortical freezing lesions. 42,43 The concomitant evaluation of PL levels showed that PE and PC were decreased by 55%, PS and PI by about 70%, while SM was unaltered. 43 These data showing inhomogeneous reduction of the individual PL can be compared with the present results indicating a more marked loss of PI and PS than of PC and PE and no change in SM level. The influence of the lipid environment on the activity of Na⁺,K⁺-ATPase has been demonstrated by the loss of enzyme activity after delipidation procedures and by the restoration of activity by the addition of well-defined lipids. Reactivation studies strongly suggest a requirement for negatively charged PL such as PS and PI. 44

Few data have been reported about the effect of ischemia on the activities of the enzymes of the glycolytic pathway. In rats, Villa et al. 45 observed lowered hexokinase and phosphofructokinase activities after 5-40 minutes of decapitation ischemia. In gerbils submitted to unilateral carotid artery occlusion for 2 hours, Djuricic et al. 46 reported a decrease in hexokinase activity, whereas phosphofructokinase and pyruvate kinase activities were unaltered. These data are in agreement with the present results showing a selective decrease in hexokinase activity. Our results showing an important loss of negatively charged PL, PI, and

### Table 4. Effect of Embolization on Cerebral Free Fatty Acids

<table>
<thead>
<tr>
<th>Fatty acid (nmol/g)</th>
<th>Control (n = 5)</th>
<th>Embolized (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic (16:0)</td>
<td>73.8 ± 4.3</td>
<td>173.7 ± 15.6*</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>40.4 ± 2.8</td>
<td>109.0 ± 9.5*</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>50.3 ± 3.2</td>
<td>150.1 ± 8.8*</td>
</tr>
<tr>
<td>Arachidonic (20:4)</td>
<td>3.6 ± 0.6</td>
<td>73.6 ± 7.5*</td>
</tr>
<tr>
<td>Docosahexaenoic</td>
<td>(22:6)</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.0 ± 7.3†</td>
</tr>
<tr>
<td>TOTAL</td>
<td>169.6 ± 6.4</td>
<td>541.4 ± 44.5*</td>
</tr>
</tbody>
</table>

Embolized rats were sacrificed 24 hours after injection of microspheres into the left internal carotid artery, and free fatty acids were measured in the left embolized hemisphere. Values are means ± SEM; n = number of animals.

* p < 0.001, † p < 0.01 between control and embolized rats.

### Table 5. Effect of Embolization on Enzyme Activities

<table>
<thead>
<tr>
<th>Enzyme activity (nmol/min/mg protein)</th>
<th>Control (n = 8)</th>
<th>Embolized (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺,K⁺-ATPase</td>
<td>122.0 ± 4.7</td>
<td>96.1 ± 10.9*</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>87.5 ± 2.2</td>
<td>75.7 ± 5.2*</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>72.5 ± 1.7</td>
<td>77.0 ± 1.9</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>242.0 ± 9.5</td>
<td>257.8 ± 12.8</td>
</tr>
</tbody>
</table>

Embolized rats were sacrificed 24 hours after injection of microspheres into the left internal carotid artery, and enzyme activities were measured in the left embolized cortex. Values are means ± SEM; n = number of animals.

* p < 0.05 between control and embolized rats.
PS, together with decreases in Na⁺,K⁺-ATPase and hexokinase activities suggest a relationship between the two phenomena, and the decreases in these enzyme activities may account for the diminutions of ATP and glucose utilizations that are observed after decapitation.

**Energy Metabolism**

Present results showing reductions in high-energy phosphates and increases in lactate and glycogen levels are comparable to those previously reported by Kogure 

et al. 47 24 hours after intracarotid injection of 35-μm microspheres. Considerable inhomogeneity of blood flow and energy metabolite levels has been reported within the hemisphere after induction of focal 48 and multifocal infarction. So, 1 hour after occlusion of the middle cerebral artery in cats, a total depletion of high-energy phosphates was found in the center of the ischemic focus, whereas adjacent areas showed variable changes in ATP, PCr, and lactate. It is to be expected that a similar metabolic picture is present after microsphere injection. The increase in lactate level denotes the persistence of anaerobic glycolysis, and the increase in glycogen content, which has been reported to occur in various forms of brain injury, 49 can be interpreted as the consequence of a decrease in glucose utilization.

Determination of metabolic levels does not give information on the cerebral metabolic rate. After induction of cerebral infarction by middle cerebral artery occlusion 50 or intracarotid microsphere injection, 52 the evaluation of glucose utilization by the deoxyglucose method evidenced closely juxtaposed regions of increased and decreased utilization. During ischemia, this method does not provide information about the brain energy consumption as it does in intact tissue because of the partial deviation of glucose metabolism toward anaerobic glycolysis. A high rate of glucose consumption does not necessarily indicate a high rate of energy use if a significant proportion of the glucose is metabolized anaerobically.

In the closed system method of Lowry et al., 12 an assumption is made that after decapitation the rate of brain energy utilization is maintained for a short time at pres ischemic levels. The technique requires rapid freezing of the cerebral tissue, so the energy metabolites were evaluated in the cortex to avoid the delay of freezing of the deep cerebral structures, which inevitably occurs after immersion of the severed head in liquid nitrogen. This method has been previously used either in non ischemic animals or in animals after transient ischemia when metabolite levels had recovered near-normal values. In the present study, the method was used after microembolization, which leads to a mix of ischemic and near-normal tissue. In areas of severe ischemia, these depleted metabolites cannot be utilized further, which represents a potential problem in applying the technique of Lowry et al to an ischemic setting. In our experimental conditions, the potential energy reserve of embolized cortex was depleted by only 16%, and its utilization after decapitation was reduced by 36%. These results can reasonably be interpreted as the consequence of a reduction in metabolic rate.

Depressions of cerebral metabolic rate have been previously reported in the recovery period following a transient ischemia. In gerbils submitted to 1 hour of unilateral carotid artery occlusion, the utilization of high-energy phosphates following decapitation was found to be decreased in the ischemic hemisphere to 20 and 50% of the control values, respectively, after 20 54 and 24 hours 55 of recirculation. Using another approach consisting of monitoring the cortical NADH fluorescence following decapitation, a lowered metabolic rate has been reported in gerbils after unilateral 56 and in rats after bilateral carotid artery occlusion. In rats with focal cortical freezing lesions, a widespread depression of glucose utilization developed with time in the adjacent cortical regions and to a lesser extent in subcortical structures. 56 The depression was significantly diminished by treatments with inhibitors of prostaglandin synthetase, suggesting that some components of the prostaglandin system were involved in the mechanism leading to the metabolic depression.

Taken together, these results indicate that mechanisms operate during ischemia to reduce the cerebral metabolic rate. Although other mechanisms such as alterations in neurotransmitters and in protein metabolism may be involved, data of the present study evidence relations between alterations of lipid metabolism and depression of the cerebral metabolic rate. PL degradation with subsequent alteration in membrane-bound enzymes and FFA release may have a direct effect on the metabolic machinery and reduce the cerebral metabolic rate.

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