Time Course of Changes in Brain Energy Metabolism of the Rat after Microsphere-Induced Cerebral Embolism

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ABSTRACT — The present study was designed to elucidate pathophysiological changes in the brain energy metabolism after cerebral ischemia. Cerebral ischemia was induced in rats by administering microspheres into the right carotid canal, and the time course of changes in cerebral energy metabolism was examined up to the 7th day after the operation. Approximately 50% of the operated rats revealed typical symptoms of stroke. In the right hemisphere, cerebral ATP and creatine phosphate of the rat on the 1st to 7th day were greatly reduced by the microsphere-induced cerebral embolism (maximally 52 and 61%, respectively), whereas the tissue lactate level was increased on the 1st, 3rd and 5th day after the embolism (maximally 125%), suggesting an induction of microsphere-induced cerebral ischemia. These changes in the tissue metabolites were accompanied by a decrease in the mitochondrial oxidative phosphorylation measured in the presence of succinate. A similar trend in the changes of biochemical markers was observed in the left hemisphere, but to a lesser degree or to an insignificant degree. The pathophysiological alterations in behavior and cerebral metabolism of microsphere-injected rats tended to return toward the normal levels on the 7th day after the operation. The results provided information on a useful model for therapeutic studies of anti-ischemic agents in the brain.

Cerebral ischemia induces various disturbances in the brain function and metabolism, which may impair physical and mental behavior in humans and animals. Even if the ischemia is transient, ischemia-induced damage is profoundly crucial in the cerebral function and metabolism. An assessment of the pathophysiology of cerebral ischemia is relevant for prophylaxis and therapy of ischemia-induced impairments in the brain function and metabolism. Numerous experimental studies have been attempted to induce pathophysiological conditions which may mimic the ischemic state in humans. Using some of these models, biochemical alterations in brain energy metabolism have been examined. For example, several investigators have shown changes in adenine nucleotides and their metabolites, glucose metabolism and mitochondrial activity after decapitation of animals (1), hypoxia induced by artificial ventilation with an oxygen-deficient gas mixture (2–5), or hypercapnia provoked by ventilation with carbon dioxide-enriched gas mixture (6–9). In general, most animals subjected to these oxygen-deficient conditions do not survive for more than one day. These experiments induce fatal or irreversible damage to the brain function and metabolism, but...
hardly provide experimental conditions that may be relevant to the therapeutic study of cerebral ischemia and infarction. The induction of incomplete, focal or regional ischemia is desirable in order to obtain data that are clinically relevant to ischemia-induced impairments in the brain function and metabolism of humans (10). Therefore, more sophisticated ischemic models are required for assessment of pathophysiology of cerebral ischemia. Recently, microsphere-induced cerebral embolism has been shown to experimentally induce focal or regional ischemia in the brain of rats, cats and rabbits (11-18). Using this type of model, the above investigators have mainly dealt with histological and behavioral changes of the animals after the brain ischemia. However, the metabolic consequences of microsphere-induced cerebral ischemia are poorly understood. The present study was designed to elucidate the extent, severity and reversibility of metabolic alterations of the brain after microsphere-induced cerebral ischemia. For this purpose, the time course of changes in cerebral energy metabolism after administration of microspheres was examined. This could provide useful information about the pathogenesis and possible prophylaxis and therapies of cerebral ischemia.

MATERIALS AND METHODS

**Induction of cerebral embolism by microspheres in rats**

Male Wistar rats, weighing 200-280 g (Charles River Japan Inc.), were used in the present study. The rats were given foods and tap water ad libitum and maintained in an animal holding room air-conditioned at 22 ± 2°C. Rats were anesthetized by intraperitoneal administration of 35 mg/kg sodium pentobarbital, and fixed in the supine position on an operation plate. After cervical incision, the right common carotid artery was isolated. The right external carotid and the right pterygopalatine arteries were ligated with strings. A polyethylene catheter (2 Fr., Atom Co., Tokyo, Japan) was inserted into the right common carotid artery. Six hundred and eighty pieces of microspheres with a diameter of 47.5 ± 0.5 μm (New England Nuclear, U.S.A.) were suspended in 150 μL of 20% dextran solution containing 0.05% Tween 80 and injected into the right common carotid artery through this cannula. The injected microspheres eventually go into the right carotid canal by this procedure. After the injection, the right common carotid artery was ligated and the wound was closed by sutures. The rats that underwent sham operation were injected with the same volume of 20% dextran solution without microspheres. Fifteen hours after the surgery, the rats were allowed to move on the plate, and their behavior was monitored.

**Behavioral observation of rats after microsphere-induced cerebral embolism**

The behavior of microsphere-injected rats was inspected 15 hours after the surgery and serially monitored at 10 o'clock in the morning for 7 days after the cerebral embolism. The rat behavior was scored on the basis of the severity of the following symptoms: paucity of movement, truncal curvature and forced circling during locomotion, which were proposed to be typical for stroke (19, 20). The score consisted of 2 (severe), 1 (moderate) and 0 (faint) for each symptom. The rats that had a total score of 5 to 6 points were considered to be the A type; 3 to 4, the B type; and less than 2, the C type.

**Preferable methods for determination of tissue metabolites**

To determine preferable methods for determination of tissue metabolites, the following methods (a to e) were examined for freezing brain tissue with liquid nitrogen. a) Rats without any pretreatment were decapitated and the brain was immediately immersed into liquid nitrogen, followed by leaving the brain in liquid nitrogen for 30 min. Then, the cerebral hemispheres were isolated. b) Rats without any pretreatment were immersed into liquid nitrogen and left for 30 min in liquid nitrogen. Then, the cerebral hemispheres were isolated.
Cerebral Ischemia and Energy Metabolism

199

c) Rats were anesthetized with ether and the skull skin of the animal was incised. The animal was immersed into liquid nitrogen and left for 30 min in the liquid nitrogen bath. Then, the cerebral hemispheres were isolated.
d) Rats were anesthetized with 1.5 l/min of N₂O, 0.5 l/min of oxygen and 1 to 4% of halothane. Halothane was inhaled at a percentage of 4% at the initial phase of anesthetization, followed by 1% halothane during the next phase. The skull skin of the animal was incised during anesthesia. At 5 min after the onset of anesthesia, the rat was immersed into liquid nitrogen and left for 30 min in the liquid nitrogen bath. Then, the cerebral hemispheres were isolated.
e) Rats were anesthetized with N₂O, oxygen and halothane as described above. The incision of the skull skin of the animal was performed during anesthesia as mentioned above. At 10 min after the onset of anesthesia, the rat was immersed into liquid nitrogen and left for 30 min in the liquid nitrogen bath. Then, the cerebral hemispheres were isolated.
f) Rats were anesthetized with N₂O, oxygen and halothane as described above. During anesthesia, the skull skin was incised. Four holes with a diameter of 4 mm were made on the skull of the animal during the anesthesia by an electric mini-grinder (No. 28400, Proxxon, West Germany). At 10 min after the onset of anesthesia, the animal was immersed into liquid nitrogen and left for 30 min in the liquid nitrogen bath. Then, the cerebral hemispheres were isolated.

Determination of tissue ATP, creatine phosphate (CP) and lactate in the hemispheres isolated as mentioned above were performed by the methods described below.

\textbf{Determination of tissue high-energy phosphates, lactate and pyruvate}

Isolation of cerebral hemispheres were performed by the e-method as described above. That is, rats were anesthetized with 1.5 l/min of N₂O, 0.5 l/min of oxygen, and halothane, and then their skull skin was incised. Ten minutes after the onset of anesthesia, the rat was immersed into liquid nitrogen and left for 30 min in the liquid nitrogen bath. Both cerebral hemispheres were isolated, and their frozen weights were precisely determined. Each hemisphere was pulverized in a stainless centrifuge tube with a stainless plunger under liquid nitrogen-cooling. The powder of brain tissue was extracted with 0.3 M HClO₄ + 0.25 mM disodium ethylenediaminetetraacetate (EDTA). After centrifugation of the extract at 1,000 x g for 20 min, the supernatant fluid was neutralized with 2.5 M K₂CO₃ and sampled for determination of tissue high-energy phosphates, lactate and pyruvate.

ATP concentration was determined according to the methods of enzymatic assay (21). Briefly, the extract was mixed with 413 mM triethanolamine, pH 7.6, 3.3 mM MgSO₄ and 5 mM glycercate-3-phosphate, 0.20 mM NADH and 7 units glyceraldehyde-3-phosphate dehydrogenase, 9 units phosphoglycerate kinase, 1.2 units of glyceral-1-phosphate dehydrogenase and 16 units of triose phosphate isomerase; and then the mixture was incubated for 10 min at 25°C. Changes in the absorbance at 340 nm were monitored. Measurements of CP were performed according to the methods of Lowry and Passonneau (22). The extract was incubated at 37°C with 100 mM Tris/HCl, pH 7.0, 0.2 mM ADP, 1.5 units of creatine kinase and 1% MgCl₂. The total ATP content in the reaction mixture was determined according to the same methods as those for determination of ATP. The CP content was determined by subtracting the initial ATP content in the extract from the total ATP content.

Tissue lactate was measured by UV methods (23). Briefly, the extract was mixed with 413 mM glycine buffer, pH 9.0, 330 mM hydrazine, 2.3 mM NAD and 13 units of lactate dehydrogenase; and then the mixture was incubated at 25°C for 1 hr. Changes in the absorbance at 340 nm were monitored. The pyruvate was also determined by UV methods (24). The extract was mixed with 90 mM potassium phosphate buffer, pH 7.4, 2.3 mM NADH and 18 units of lactate dehydrogenase and incubated at 25°C for 15 min. Changes in the absorbance at 340 nm were monitored.
In the present study, the concentrations of tissue metabolites were expressed as \( \mu \) moles/g of dry tissue. The ratio of dry tissue weight to the frozen tissue weight was estimated in a preliminary study and found to be 0.200 ± 0.002 (n = 10). The ratio of microsphere-injected rat brains was 0.196 ± 0.004 (n = 5), indicating an insignificant difference between two groups.

Isolation of cerebral mitochondria

Isolation of the cerebral mitochondria was carried out according to the methods of Sordahl et al. (25). Briefly, the brain was removed within 15 sec after decapitation and immersed in a cold 0.3 M mannitol - 0.1 mM EDTA solution, pH 7.4 (ME buffer). The cerebral hemispheres were separated in the cold ME buffer, and the mitochondria from both sides were isolated separately. It should be mentioned that separation of cerebral hemispheres and the following isolation of mitochondria were performed using single brain. After determining the wet weight, each hemisphere was homogenized with a loosely fitted glass-Teflon homogenizer. The homogenate was centrifuged at 600 × g for 8 min at 0–4°C. The supernatant fluid was centrifuged at 10,000 × g for 10 min. The pellet was rehomogenized in the ME buffer, and then centrifuged at 5,000 × g for 10 min. After decantation of the supernatant fluid, the pellet was washed with 20 ml of ME buffer and reserved as the mitochondrial fraction. The washing solution and the supernatant fluid as described above were combined, and the resulting suspension was centrifuged at 5,000 × g for 10 min. The collected pellet and the pellet reserved as above were suspended in ME buffer or 100 mM potassium phosphate - 0.5% bovine serum albumin and sampled for determination of oxidative phosphorylation activity or succinate dehydrogenase activity of the cerebral mitochondria, respectively. The supernatant fluid was further centrifuged at 20,000 × g for 20 min. The resulting supernatant fluid was used for determination of cytoplasmic enzyme activities such as phosphofructokinase and pyruvate kinase as described later. Mitochondrial protein concentrations were determined by the methods of Lowry et al. (26). The protein yields of the mitochondria from the right and the left cerebral hemisphere were 5.6 ± 0.2 and 5.7 ± 0.2 mg protein/g wet tissue, respectively (n = 56). There were no significant differences in the protein yields of mitochondria from rats with and without administration of microspheres.

Measurements of the oxidative phosphorylation ability

Measurements of the oxidative phosphorylation ability of the isolated cerebral mitochondria were performed within one hour after the isolation according to the methods of Sordahl et al. (25). Oxygen consumption of the mitochondria was continuously monitored at 25°C on an oxygraph with a Clark type DO/O2 electrode (Oxygraph-8, Central Sciences Co., Tokyo). The reaction mixture contained 0.3 M mannitol, 10 mM KCl, 10 mM Tris/HCl, 5 mM K2HPO4, 0.2 mM EDTA and about 1.0 mg mitochondrial protein. Either 10 mM potassium glutamate or 10 mM potassium succinate was used as a substrate for the phosphorylation. State 3 respiration was initiated by addition of 250 nmol ADP. As indicators for the oxidative phosphorylation ability of the mitochondria, the following parameters were observed: respiratory control index (RCI: ratio of oxygen consumption at state 3 to that at state 4), ratio of ADP consumed to oxygen consumed at state 3 (ADP/O), rate of oxygen consumption at state 3 (Q03), rate of oxygen consumption at state 4 (Q04), oxidative phosphorylation rate (OPR: specific rate of ATP produced at state 3).

Reagents and enzymes

Disodium ATP, disodium ADP, phosphoenolpyruvate, and alpha-glycerophosphate dehydrogenase and triose-isomerase were purchased from Sigma (St. Louis, U.S.A.) and NAD, NADH, NADP, glucose 6-phosphate dehydrogenase, hexokinase, lactate dehydrogenase, creatine kinase were from Boehringer
Statistics
Values in the text represent the mean ± S.E.M. Activities obtained from rats without operation were used as the standard (non-operated group). For the statistical tests, Student's t-test was used for comparison of the values for the microsphere-injected group with those for the sham-operated group or the non-operated group. Analysis of variance, followed by Dunnett's t-test was used for comparison of the time course of changes in the microsphere-injected group and the non-operated group, respectively. A confidence level of more than 95% was taken to be statistically significant (P < 0.05).

RESULTS

Induction of cerebral embolism
In the present study, we injected microspheres into 155 rats. Forty-two rats died within 24 hours after the operation (27%). Seventy-nine rats exhibited typical symptoms of stroke such as truncal curvature and forced circling during locomotion (A type, 51%). However, among these animals, 6 rats died before a biochemical study was performed (4%). Eventually, 73 rats were used for biochemical study at an appropriate experimental sequence. There were 25 rats that exhibited symptoms similar but slightly milder than those of the A type (B type, 16%). Nine rats showed such behavior to a minimal degree (C type, 6%). Postmortal examination of the brain of rats that died within 24 hours of the operation revealed loss of tightness of the brain tissue and marked edema, suggesting a critical damage to regulation of ionic constituents in brain cells by injection of microspheres. In the present study, we did not use the rats with B or C type of symptoms of stroke in order to avoid variations of behavioral and metabolic alterations during the following experimental period as much as possible. All rats that underwent a sham operation (54 rats) survived during the experimental period. In addition, 24 rats without any operation were used for biochemical study as the standard. Another 70 rats without operation were used for a pilot study on determination of tissue metabolites.

Behavioral changes of operated rats
In the first set of experiments, time courses of behavioral changes were examined and the results are shown in Fig. 1. The symptomatic behavior of A type rats gradually improved with time after the operation. Six days after the operation, the behavioral recovery was considerably enhanced. Rats that underwent sham operation did not show any symptoms for stroke throughout the experimental period.

Preferable freezing method for determination of tissue metabolites
The cerebral ATP, CP and lactate contents were determined according to the 6 methods (a to f) as described in the Methods section (Table 1). The cerebral ATP and CP were appreciably higher in the d-, e- and f-methods than in the a-, b- and c-methods. In the latter cases, animals were not anesthetized and their
Table 1. Cerebral metabolites of rats subjected to various pretreatments before sacrifice

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Numbers of experiments</th>
<th>ATP</th>
<th>CP</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Decapitated rats without any treatment</td>
<td>10</td>
<td>12.92 ± 0.11</td>
<td>13.78 ± 0.33</td>
<td>24.13 ± 0.65</td>
</tr>
<tr>
<td>b) Immersed rats without any treatment</td>
<td>10</td>
<td>13.52 ± 0.16</td>
<td>14.86 ± 0.21</td>
<td>16.70 ± 0.65*</td>
</tr>
<tr>
<td>c) Rats with skull-incisioned</td>
<td>14</td>
<td>15.29 ± 0.25*</td>
<td>16.83 ± 0.26*</td>
<td>11.97 ± 1.25*</td>
</tr>
<tr>
<td>d) Rats with 5 min-anesthesia</td>
<td>8</td>
<td>17.31 ± 0.23*</td>
<td>19.40 ± 0.40*</td>
<td>15.65 ± 2.28*</td>
</tr>
<tr>
<td>e) Rats with 10 min-anesthesia</td>
<td>14</td>
<td>17.61 ± 0.39*</td>
<td>19.65 ± 0.27*</td>
<td>15.62 ± 1.03*</td>
</tr>
<tr>
<td>f) Rats with 10 min-anesthesia and skull-bolting</td>
<td>14</td>
<td>18.20 ± 0.14*</td>
<td>21.65 ± 0.17*</td>
<td>21.59 ± 1.64*</td>
</tr>
</tbody>
</table>

Values are expressed as μmoles/g dry tissue. Each value represents the mean ± S.E.M. *Significantly different from the values of the a) group (P < 0.05).

The cerebral skin was not incised. In contrast, the cerebral lactate concentration was lower in the b-, c-, d- and e-methods than in the a- and f-methods. The total contents of high-energy phosphates for brain tissue, when determined by the e-method, were comparable to the reports of other investigators who used the freeze-blowing or funnel-freezing method (27, 28). We also confirmed in a pilot study that either a further period of anesthesia (20 min) or inhalation with 2:1 ratio of N2:O2 reduced tissue high-energy phosphates and increased tissue lactate.

Cerebral lactate concentration

Lactate contents of cerebral hemispheres isolated from rats after microsphere-induced cerebral embolism were determined, and the results are shown in Fig. 2. The lactate content markedly increased in the right hemisphere isolated on the first day after microsphere-induced cerebral embolism (126% increase). The raised level of lactate content was also observed in the right hemisphere on the 3rd and the 5th days after the surgery (62 and 52% increases, respectively). However, the lactate concentration of the right hemisphere returned to the initial level on the 7th day af-

![Fig. 2. Time course of changes in lactate concentrations of the right (●) and left (■) hemispheres after microsphere-induced cerebral embolism. (○) and (□) represent those of the right and left hemispheres of sham-operated rats, respectively. Values represent the mean ± S.E.M. Numbers of experiments for microsphere administered rats were 17 (no operation) and 9 (each on the 1st, 3rd, 5th and 7th days after the operation), whereas those for sham-operated rats were 7 (on the 1st day), 8 (on the 3rd day), 7 (on the 5th day) and 6 (on the 7th day). *Significantly different from the initial value (P < 0.05).](image-url)
After the operation. In the left hemisphere, the lactate concentrations were essentially unchanged except that the lactate concentration on the 3rd day after the operation was slightly increased. The lactate concentrations of both hemispheres of sham-operated rats were not altered throughout the experimental period.

The pyruvate contents of the both hemispheres were also determined simultaneously. The control pyruvate contents of the right and left hemispheres were $1.36 \pm 0.16$ and $1.47 \pm 0.16 \mu$moles/g dry tissue, respectively ($n = 17$). The pyruvate contents after the embolism tended to increase slightly, but insignificantly (data not shown).

**Tissue ATP and CP contents**

Cerebral ATP and CP contents were measured after microsphere-induced cerebral embolism, and the results are shown in Fig. 3a and 3b, respectively. A marked reduction of the ATP content (52% decrease) was seen on the 1st day after the operation. Significantly reduced levels of ATP concentration in the right hemisphere were also observed on the 3rd, 5th and 7th days after the operation (49, 39, and 28% decreases, respectively). This also showed that the ATP levels tended to be restored with time after the operation. A similar trend in changes of the ATP concentration was seen in the left hemisphere, but to a lesser degree.

A marked reduction of the CP content in the right hemisphere was observed soon after the embolism; large reductions in the CP contents were seen on the 1st, 3rd and 5th days (59, 61 and 46% decreases, respectively). As with the ATP content, the CP concentration of the right hemisphere tended to recover toward the initial value with time after the operation. The CP level of the left hemisphere after the cerebral embolism was also decreased, but to a lesser degree than that of the right hemisphere. The ATP and CP levels of both hemispheres isolated from sham-operated rats were slightly, but significantly lower than the initial.

![Fig. 3](image-url) Time course of changes in ATP (a) and creatine phosphate (b) concentrations of the right (●) and left (■) hemispheres after microsphere-induced cerebral embolism. (○) and (□) represent those of the right and left hemispheres of sham-operated rats, respectively. Values represent the mean ± S.E.M. Numbers of experiments for microsphere administered rats were 17 (no operation) and 9 (each on the 1st, 3rd, 5th and 7th days after the operation), whereas those for sham-operated rats were 7 (on the 1st day), 8 (on the 3rd day), 7 (on the 5th day) and 6 (on the 7th day). *Significantly different from the initial value ($P < 0.05$).
Oxidative phosphorylation activity of the cerebral mitochondria

In another set of experiments concerning the isolated mitochondria, their oxidative phosphorylation activity was determined (Fig. 4). The OPR has been proposed to be a marker for the ischemic state (29). The OPR of the right hemisphere was markedly reduced on the 1st day after the operation when measured both in the presence of glutamate and succinate (55 and 56%, respectively). The reduced activity of the OPR of the right hemisphere in the presence of succinate lasted up to the 7th day after the operation, although the OPR tended to recover toward the initial level with time after the operation. In contrast, the OPR of the right hemisphere, when measured in the presence of glutamate, exceeded the initial value on the 3rd, 5th and 7th day after the operation. The OPR of the left hemisphere was slightly decreased only on the 1st day after the operation when measured in the presence of succinate or glutamate (11 or 7%, respectively). There were no substantial decreases in the OPR activity of the right and left hemispheres of sham-operated rats during the experimental period.

As an index of mitochondrial ATP producing ability after cerebral embolism, several other indicators of oxidative phosphorylation activity of the mitochondria isolated on the 3rd day after operation were measured (Table 2). As described above, the mitochondrial OPR of the right hemisphere was significantly reduced when measured in the presence of succinate, while it was increased when monitored in the presence of glutamate. The reduction of the OPR in the presence of succinate was accompanied by significant decreases in $Q_{03}$ and $Q_{04}$ and a trend of decrease in RCI. The increment in the OPR in the presence of glutamate was accompanied by significant increases in RCI, ADP/O and $Q_{03}$.

DISCUSSION

In the present study, we have produced cerebral embolism by administering 680 microspheres with a diameter of 48 $\mu$m into the rat carotid canal. Approximately 50% of the
Table 2. Oxidative phosphorylation ability of the mitochondria isolated from the right and left hemispheres on the 3rd day after microsphere-induced cerebral embolism in rats

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>sham operation</th>
<th>microsphere injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCI right</td>
<td>3.36 ± 0.11</td>
<td>3.27 ± 0.12</td>
<td>3.64 ± 0.14*</td>
</tr>
<tr>
<td>left</td>
<td>3.44 ± 0.11</td>
<td>3.35 ± 0.14</td>
<td>3.12 ± 0.11</td>
</tr>
<tr>
<td>ADP/O left</td>
<td>2.53 ± 0.05</td>
<td>2.44 ± 0.03</td>
<td>2.66 ± 0.03</td>
</tr>
<tr>
<td>right</td>
<td>2.55 ± 0.04</td>
<td>2.50 ± 0.04</td>
<td>2.55 ± 0.04</td>
</tr>
<tr>
<td>QO3 right</td>
<td>34.5 ± 0.8</td>
<td>37.1 ± 0.1</td>
<td>41.0 ± 1.6*</td>
</tr>
<tr>
<td>left</td>
<td>36.3 ± 1.8</td>
<td>37.9 ± 1.2</td>
<td>37.9 ± 1.3</td>
</tr>
<tr>
<td>QO4 right</td>
<td>10.3 ± 0.3</td>
<td>11.5 ± 0.5</td>
<td>10.7 ± 0.5</td>
</tr>
<tr>
<td>left</td>
<td>10.3 ± 0.4</td>
<td>11.9 ± 0.5</td>
<td>12.2 ± 0.5</td>
</tr>
<tr>
<td>Succinate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP/O right</td>
<td>2.63 ± 0.05</td>
<td>2.62 ± 0.08</td>
<td>2.46 ± 0.14</td>
</tr>
<tr>
<td>left</td>
<td>2.57 ± 0.05</td>
<td>2.83 ± 0.09</td>
<td>2.62 ± 0.06</td>
</tr>
<tr>
<td>QO3 right</td>
<td>1.92 ± 0.03</td>
<td>1.92 ± 0.03</td>
<td>1.86 ± 0.03</td>
</tr>
<tr>
<td>left</td>
<td>1.91 ± 0.03</td>
<td>1.96 ± 0.02</td>
<td>1.98 ± 0.02</td>
</tr>
<tr>
<td>QO4 right</td>
<td>107.1 ± 3.7</td>
<td>104.0 ± 5.7</td>
<td>81.2 ± 3.7*</td>
</tr>
<tr>
<td>left</td>
<td>111.1 ± 7.2</td>
<td>109.5 ± 3.6</td>
<td>108.1 ± 5.4</td>
</tr>
<tr>
<td>RCI left</td>
<td>40.7 ± 0.8</td>
<td>39.8 ± 2.2</td>
<td>33.2 ± 0.8*</td>
</tr>
<tr>
<td>right</td>
<td>41.3 ± 1.3</td>
<td>39.0 ± 1.8</td>
<td>40.9 ± 1.8</td>
</tr>
</tbody>
</table>

The following units were used: RCI (respiratory control index: ratios of oxygen consumption at state 3 to that at state 4), ADP/O (nanomoles ADP consumed at state 3/nanomols oxygen), QO3 (nanomols oxygen consumed at state 3/mg protein/min). QO4 (nanomols oxygen consumed at state 4/mg protein/min). Each value represents the mean ± S.E.M. of 6 to 7 experiments. Control values were obtained in non-operated rats. *Significantly different from the value for sham-operated rats (P < 0.05).

Rats revealed typical symptoms of stroke when inspected on the first day after the surgery. As described in the Introduction, several investigators have demonstrated microsphere-induced cerebral embolism. However, there was very little information concerning the rate of success of this operation for induction of cerebral ischemia or infarction. In this sense, the experimental procedure in the present study may be meaningful. Although observed behavioral changes of the microsphere-injected rats do not always parallel changes in biochemical activity induced by an embolism, the brain energy metabolism of these rats was profoundly altered after the embolism.

In a pilot study, we attempted to elucidate optimal freezing conditions for determination of labile cerebral metabolites. As shown in Table 1, the highest contents of brain high-energy phosphates were obtained in rats with N2O-O2-halothane anesthesia and skull holing (the f-method), and the e-method yielded higher contents of cerebral high-energy phosphates than the a- to c-methods in which rats were not anesthetized. However, the f-method yielded a higher cerebral lactate level relative to the e-method in which rats were treated with 10 min-N2O-O2-halothane anesthesia. There are two sophisticated methods for freezing brain tissue with liquid nitrogen for the studies of labile cerebral metabolites, that is, freeze-blowing method under conscious but immobilized conditions in a special restraining cage (27) and the funnel-freezing method under vinylether-anesthesia (28). Brain ATP, CP and lactate levels by the former method were 2.45, 4.05 and 1.23 μmoles/g frozen tissue, and those by the latter method, 3.00, 4.71 and 1.64 μmoles/g frozen tissue, respectively. Since the ratio of frozen tissue to dry tissue was about 5, our value for ATP was slightly higher and that for CP slightly lower than those obtained by the two methods. That is, our value for the total brain high-energy phosphates (ATP + CP) was almost similar to those reported by these two groups. The difference in tissue lactate levels may be due to differences in the detecting methods em-
ployed, since the ratio of lactate to pyruvate in the present study (about 11) was approximately similar to theirs (13.5 to 13.6). Thus, the e-method in the present study is practically comparable to the two approved methods in terms of determination of cerebral labile metabolites such as ATP, CP and lactate. The results in the pilot study motivated us to use the e-method for determination of cerebral metabolites of microsphere-injected rats.

The lactate concentration of the right hemisphere on the 1st day after the embolism was markedly increased, and the level remained high on the 3rd and 5th days. It should be noted that the lactate level was measured using the whole cerebral hemisphere in the present study. Furthermore, we microscopically observed heterogenous distribution of microsphere-induced histological damage to the forebrain area (data not shown). This suggests that the lactate concentration in ischemic areas is even greater than the level measured here. Since lactate concentration is well-recognized to increase when the tissue is ischemic (1, 10, 30-32), the results suggest that the right hemisphere remains ischemic for at least 5 days after the embolism. In contrast, the lactate concentration of the left hemisphere was not increased by the embolism, indicating that the left brain is not severely ischemic over the experimental period monitored.

Regardless of the degree of the sustained ischemic state in either hemisphere, the ATP and CP contents of the both hemispheres were, markedly reduced during the experimental period ranging from the 1st to 5th day after the operation. The reduction of tissue high-energy phosphate levels is well-recognized to be indicative of an ischemic or oligemic state in the tissue (1, 32, 33). The findings suggest that microsphere-induced cerebral embolism in the present study provokes considerably long-term ischemia and that this ischemia induces severe impairments in energy producing ability in the brain. However, this severe impairment in the energy metabolism appears to be somewhat restored on the 7th day after the embolism. That is, microsphere-induced changes in lactate, pyruvate, ATP and CP were reversed completely or appreciably by the 7th day. Similarly, such recovery was also seen in changes of mitochondrial oxidative phosphorylation ability of the right hemisphere. These results suggest that ischemic changes in cerebral energy metabolism induced by administration of microspheres in the present study are biochemically reversible seven days or later after the surgery. In a preliminary study, we observed that when the numbers of microspheres used were increased to more than 900 (900 – 1,200), the mortality of operated rats were increased or more severe damage to the brain energy metabolism occurred (34). Thus, this method of microsphere-embolism is capable of producing reversible or irreversible ischemia in the brain depending upon the numbers of microspheres employed. Kogure et al. (11) have shown decreases in ATP and CP in the rat brain receiving a massive amount of microspheres (3000 pieces of 50μm microsphere) after 24 hours of operation. Our findings are comparable to this and provided a further evidence for a long-lasting shortage of cerebral high-energy phosphates in the ischemic brain.

As a further examination of brain energy metabolism during microsphere-induced cerebral embolism, we examined cerebral mitochondrial ability to produce ATP in the ischemic brain. For this purpose, time course of changes in mitochondrial biochemical activities such as oxidative phosphorylation ability in the electron transfer system were determined. The OPR, ATP producing ability in the mitochondria, in the presence of succinate was decreased throughout the experimental period by the embolism; the rate was most profoundly depressed on the 1st day and appreciably restored on the 7th day. The results suggest that microsphere-induced cerebral embolism results in severe impairment of the mitochondrial ATP production. As shown in Table 2, in which several parameters of the oxidative phosphorylation ability on the 3rd day after the embolism are listed, the depression in the OPR determined in the presence of
succinate was accompanied by a decrease in RCI and O$_{2}$$_{2}$, but not by a reduction in ADP/O, suggesting the embolism substantially decreases oxygen consumption at state 3.

It is interesting that the OPR of the right hemisphere tended to increase when glutamate was used as a substrate, except for that on the first day which was found to be markedly declined, whereas the OPR was significantly decreased when succinate was used as a substrate. This implies that the ATP producing ability through the FADH$_{2}$ system in the mitochondria is inhibited by the cerebral embolism, whereas that through the NADH system is enhanced. Generally, tissue NADH is increased during ischemia (35, 36). The enhancement of ATP producing ability through the NADH system is presumably related to this increase in NADH. The results also suggest that there is an adaptive mechanism through the NADH system under pathophysiological conditions by which the failure in the ATP production through the FADH$_{2}$ system is compensated. Such an adaptive response has been shown to occur in the mitochondrial oxidative phosphorylation ability of rats exposed to hypoxic insults (37). Thus, induction of an adaptive mechanism seems to be characteristic in brain energy metabolism under pathophysiological conditions by which the failure in the ATP production through the FADH$_{2}$ system is compensated. Such an adaptive response has been shown to occur in the mitochondrial oxidative phosphorylation ability of rats exposed to hypoxic insults (37).

Hence, it would be difficult to compare the severity and extent of the ischemic injury induced in the present study with theirs. Presumably, pathophysiological damage in their study appears to be more severe than ours, because we found mild edema in the right hemisphere of rats that survived for 3 to 7 days after the operation. Thus, this microsphere-induced cerebral embolism may be a useful method for assessment of biochemically reversible pathophysiological alterations of ischemic brain and thus suitable for therapeutic study of brain ischemia. It is unlikely that embolism-induced nerve cell damage is histologically reversed after long-lasting cerebral ischemia. Presumably, behavioral and biochemical recovery of microsphere-injected rats is due to a compensatory mechanism of undamaged nerve cells as a result of the healing process.

In summary, the present study has shown changes in brain energy metabolism after microsphere-induced cerebral embolism. Administration of microsphere induced ischemic derangements in the cerebral energy metabolism which lasted at least 5 days and partially recovered 7 days after the embolism. The cellular basis for microsphere-embolism induced metabolic disturbances in energy production may be attributed to the mitochondria.
drial oxidative phosphorylation relating to the FADH₂ system.

REFERENCES


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