To assess the effect of a glucose infusion on brain extracellular fluid (ECF) during systemic hypoxia, changes in glucose and lactate concentrations in cerebral ECF during and after moderate hypoxic hypoxia were measured in adult, conscious, unrestrained rats, with a microdialysis probe in the posterior hippocampus. The rats were given either saline (n = 6) or 50% glucose solution (n = 6) for 3 h, starting 60 min before the onset of hypoxia. Hypoxia was produced by circulating 7% O₂ gas in a plastic chamber for 90 min. In saline-infused animals, brain ECF glucose concentrations decreased slightly during hypoxia, although blood glucose concentrations did not change. Blood lactate concentrations increased to 6.28 ± 0.91 mM, at 60 min after the onset of hypoxia (P < 0.05). Brain ECF lactate concentrations increased to 3.53 ± 0.20 mM and remained constant during 60 min of steady-state hypoxia (P < 0.05) and decreased to the basal level within 60 min after the end of hypoxia. When sodium lactate solution was infused intravenously for 90 min (n = 4), blood lactate concentrations increased to a level as high as those found during hypoxia. However, the brain ECF lactate concentration increased only to 1.86 ± 0.08 mM. In glucose-infused animals, the blood glucose concentration reached 339.1 ± 32.3 mg/dl at the end of the glucose infusion, and the brain ECF glucose concentration increased to 54.7 ± 7.3 mg/dl. Although the blood lactate concentration during hypoxia was significantly higher in the glucose-infused group than in the saline-infused group, there was no significant difference in the brain ECF lactate concentrations between these groups. In conclusion, the current study suggests that glucose supply is not a rate-limiting factor for anaerobic glycolysis in the brain, and the lactate produced in brain tissue seems to be used within the brain during and after a period of moderate hypoxia. (Key words: Brain: hippocampus. Metabolism: glucose. Oxygen: hypoxic hypoxia.)

**Under anaerobic conditions**, lactic acid is produced as the major glycolytic end-product in various tissues. When one begins to breathe O₂ again after a period of anaerobic metabolism, the lactic acid is converted to glucose, mainly in the liver, or it is used directly for energy in tissues such as the heart and other muscles. Prolonged accumulation of lactic acid under anaerobic conditions can cause severe metabolic acidosis, which sometimes damages physiologic functions irreversibly.

---

* Postgraduate student.
† Lecturer, Department of Anesthesiology.
‡ Assistant, Department of Anesthesiology.

Received from the Department of Anesthesiology, Kyoto Prefectural University of Medicine, Kyoto, Japan. Accepted for publication May 26, 1992.

Address reprint requests to Dr. Harada: Department of Anesthesiology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirakoji, Kamigyoku, Kyoto, 602, Japan.

In the brain, lactate production begins to increase and cerebral tissue pH begins to decrease when PaO₂ = 45–50 mmHg.¹ Mitochondrial respiration cannot be supported when PaO₂ < 25 mmHg and consciousness is lost because of a rapid depletion of high-energy organic phosphates.¹ However, experimental studies indicate that energy metabolism remains normal during mild or moderate hypoxia (PaO₂ = 25–40 mmHg), probably because glycolysis is stimulated to a sufficient degree to maintain normal tissue adenosine triphosphate concentrations.² In previous studies, brain concentrations of metabolic substances have been estimated by measurement of the concentrations in cerebrospinal fluid or frozen tissue.³⁴

Recently, microdialysis has been used to study changes in extracellular fluid (ECF) concentrations of various substances over time, including glucose and lactate.⁵⁷–⁸ Kuh et al. have shown that electroconvulsive shock rapidly increases brain tissue concentrations of lactate. This is followed by a delayed (several minutes) increase in brain ECF lactate concentrations, when energy supplies could maintain membrane integrity and function.⁷ This indicates that the intracellular concentrations of lactate can be estimated by measurement of the ECF level in a steady state.

In the current study, we used microdialysis to measure hypoxia-induced changes in lactate metabolism in the brains of conscious rats. Our special concern in this study is the extent to which anaerobic glycolysis is stimulated in the brain during steady-state hypoxia and how long it takes for the lactate concentration to return to normal after the hypoxic episode. A steady state of moderate hypoxia (PaO₂ = 27–32 mmHg for 60 min) was induced by allowing the rats to inhale 7% O₂ gas for 90 min.

A second goal is to determine whether a glucose load will increase lactate accumulation in the brain during hypoxia. Because glucose and lactate are transported via specific carriers on the blood–brain barrier (BBB),⁸ glucose infusion may have different effects on lactate metabolism in the brain and blood during hypoxia. In previous studies, it has been shown that a glucose load can worsen

---

neuronal damage resulting from cerebral ischemia, probably by causing accelerated production of lactic acid in brain tissue.\textsuperscript{9-11} In the current study, to ascertain whether such a phenomenon also occurs during hypoxia, one group of rats was given 50% glucose solution before the onset of hypoxia. To determine where the lactate in the brain ECF came from and where it went, we also measured the amount of blood-borne lactate transported through the BBB in the rats given a highly concentrated lactate solution.

**Materials and Methods**

**PREPARGATIONS**

This protocol was reviewed and approved by the Institutional Animal Care and Use Committee. Male Wistar rats weighing 220–280 g were used. Three days before the experiment, each animal was anesthetized with pentobarbital (50 mg/kg, intraperitoneally) and fixed in a stereotaxic frame. The skull was exposed, and a hole was drilled in the right temporal bone. A guide cannula was implanted in the hippocampus (the tip of the cannula was placed 5.4 mm caudal, 5.0 mm lateral, and 4.0 mm ventral to the bregma) and cemented onto the skull with dental acrylic resin (Nissin Dental Products, Kyoto, Japan). On the day before the experiment, each rat was anesthetized again, and polyethylene cannulae were inserted into the femoral artery and vein. The cannulae were advanced subcutaneously to the posterior neck and fixed. All rats were allowed to recover in individual cages under controlled lighting and temperature, and they had free access to commercial food pellets and water until the day of the experiment. All experiments were performed from 12:00 noon to 6:00 P.M. After the hypoxia experiment, the rats were allowed to recover in individual cages, where their behavior was observed. Those that survived for 24 h were killed, and the postmortem examinations confirmed the locations of the guide cannulae.

**EXPERIMENTAL PROCEDURE**

Every animal was conscious and allowed to move freely in a plastic chamber during the experiment.

**Hypoxia**

Each animal was placed in a plastic chamber with a 7-l capacity, which had an inlet and outlet for gas flow. The arterial cannula was connected to a pressure transducer to record mean arterial pressure. Heart rate was measured with a cardiostethometer triggered by the blood pressure pulse. Respiratory rate was counted by an observer. The venous cannula was connected to a syringe pump to infuse either saline (saline-infused group) or a 50% glucose solution (glucose-infused group). The microdialysis probe was implanted through the guide cannula into the hippocampus. Then the chamber was closed, and compressed air was circulated at 31/min.

One hour after the beginning of the experiment, saline or 50% glucose solution was infused for 3 h, as shown in figure 1. The infusion rate was 8.3 \( \mu \)l/min \( \cdot 100 \text{ g}^{-1} \). After 1 h of infusion, the air in the chamber was replaced with a mixture of 7% O\(_2\)-93% N\(_2\) for 90 min. The O\(_2\) concentration in the chamber was monitored with an O\(_2\) analyzer (MiniOX I, Catalyst Research, Owings Mills, Maryland). Then the chamber was opened to air, and the measurements were continued in the open chamber for another 90 min.

**Sodium Lactate Infusion**

The venous cannula was connected to a syringe pump so that 3.6 M sodium lactate solution could be infused, after the pH had been adjusted to 7.4 with NaOH. The microdialysis probe was implanted through the guide cannula into the hippocampus. One hour after the beginning of the experiment, 3.6 M sodium lactate solution was infused at 8.3 \( \mu \)l/min \( \cdot 100 \text{ g}^{-1} \) for 90 min.

**MICRODIALYSIS SYSTEMS AND GLUCOSE AND LACTATE ANALYSIS**

The CMA/10 Microdialysis Probe (Carnegie Medicine, Stockholm, Sweden) (diameter, 0.5 mm; length, 2 mm) was used. With a CMA/100 pump, the probe was perfused with artificial cerebrospinal fluid (millimolar: NaCl 126.5, KCl 2.4, KH\(_2\)PO\(_4\) 0.5, CaCl\(_2\) 1.1, MgCl\(_2\) 1.1, Na\(_2\)SO\(_4\) 0.5, and N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid-Na 20, pH = 7.3) at 2 \( \mu \)l/min.

![Blood glucose concentrations before, during, and after hypoxic hypoxia in glucose- and saline-infused animals; n = 6 for all data points. All values are mean ± SE. *P < 0.05 versus control value in each group. **P < 0.05 versus saline-infused group.](image-url)

**FIG. 1.** Blood glucose concentrations before, during, and after hypoxic hypoxia in glucose- and saline-infused animals; n = 6 for all data points. All values are mean ± SE. *P < 0.05 versus control value in each group. **P < 0.05 versus saline-infused group.
Before the experiment, the probe was calibrated in artificial cerebrospinal fluid containing 50 mg/dl glucose and 1.0 mM lactate, and the recovery rates of the probe for these substrates were obtained. The temperature of the fluid was maintained at 36°C. The average in vitro recovery rates of the microdialysis probes for glucose and lactate were approximately 10% and 15%, respectively. The microdialysis probe was perfused in vivo for at least 60 min before a sample was taken, so that stable glucose and lactate concentrations could be obtained. Microdialysis samples were collected from each rat every 30 min. The brain ECF glucose and lactate concentrations were calculated by dividing the concentrations in the perfusate by the respective recovery rates.

Blood samples were drawn from the arterial cannula every 30 min to measure arterial blood gases and blood lactate and glucose concentrations. Glucose and lactate concentrations were measured with a glucose analyzer (model 23A) and lactate analyzer (model 23L, Yellow Springs Instruments, Yellow Springs, Ohio), respectively. With the analog recorder outputs, these analyzers were calibrated in standard solutions and the sensitivities confirmed to be 0.1 mg/dl for glucose and 0.01 mM for lactate. Then the programs of the digital panels of these devices were modified to display the lower limits of detection.

STATISTICS

Statistical analysis was performed for the hypoxia experiment with an analysis of variance (three-factor) with repeated measures. When a statistically significant difference in group–time interaction, group effect, or time effect was found, the analysis was continued by calculation of contrasts between individual pairs of groups at each time point, between individual pairs at the first time point (the basal value), and at a certain other time point in each group with the Bonferroni correction for multiple comparisons (figs. 1–4). Likewise, the data from the sodium lactate infusion experiment were evaluated with analysis of variance (two-factor) with repeated measures, followed by the Bonferroni correction.
To compare the blood and brain ECF lactate concentrations among the lactate infusion and hypoxia groups, Student's *t* test was used. Differences with *P* < 0.05 were considered significant.

**Results**

**Hypoxia**

The O₂ concentration in the chamber decreased immediately after the circulating gas was switched from air to 7% O₂–93% N₂ and it stabilized within the first measurement period. Therefore, the hypoxic period (90 min, three measurement periods) was divided into an initial phase (30 min, one measurement period) and a steady-state phase (60 min, two measurement periods). Most of the rats were excited and struggled to escape from the chamber for a moment after the hypoxic gas was infused into the chamber. Later, they all squatted down, and responses to stimulation (sound) were rare. Three rats in the glucose-infused group and two in the saline-infused group died during hypoxia, after irregular deep breathing, abrupt bradycardia, and hypotension. However, there were no convulsions in any of the rats during the hypoxia. Data from the six surviving rats in each group were analyzed.

Values of hemodynamic and respiratory variables before, during, and after hypoxia are shown in table 1. There were no significant differences between the saline- and glucose-infused groups in any of the physiologic variables measured throughout the experimental period. *P*aco₂ had decreased to 26.7 ± 1.5 mmHg and 27.5 ± 1.4 mmHg by 30 min after the hypoxia began in the saline- and glucose-infused rats, respectively. The *P*aco₂ remained low until the end of the hypoxic period (90 min after the beginning of hypoxia). Hyperventilation (70% increase in respiratory frequency) occurred during hypoxia, resulting in significant reductions in *P*aco₂ to 17.1 ± 1.7 mmHg and 17.4 ± 1.4 mmHg, in saline- and glucose-infused rats, respectively, and *P*aco₂ remained low throughout the hypoxic period. Blood *pH* first increased, then gradually decreased, during hypoxia. Blood pressure decreased and heart rate increased significantly during hypoxia. By 90 min after the end of hypoxia, blood pressure, respiratory rate, *pH*, and *P*aco₂ returned to the basal values. However, heart rate remained at values similar to those measured during hypoxia, and *P*aco₂ increased to levels higher than the basal levels.

As shown in figure 1, the blood glucose concentration did not change in animals given saline, but it reached 339.1 ± 32.3 mg/dl at the end of the infusion in animals given glucose. Basal brain ECF glucose concentrations in the saline- and glucose-infused groups were 20.6 ± 1.3 mg/dl and 20.8 ± 2.8 mg/dl, respectively, and they decreased significantly in animals given saline but returned to the basal level after the animals breathed normal air. In contrast, the brain ECF glucose concentration reached 54.7 ± 7.3 mg/dl by the end of the glucose infusion, and then it decreased (fig. 2). As shown in figure 3, the blood lactate concentration in the glucose-infused group increased to 10.76 ± 0.77 mM by 60 min after the onset of hypoxia, which was significantly higher than that measured in the saline-infused group (6.28 ± 0.91 mM). The blood lactate level in the saline-infused group returned to the basal level by the end of the experiment, whereas it remained higher than the basal level in the glucose-infused group. Brain ECF lactate concentrations increased from 1.37 ± 0.07 mM to 3.53 ± 0.20 mM in the saline-infused group, and from 1.32 ± 0.08 mM to 3.82 ± 0.28 mM in the glucose-infused group, respectively, but there were no significant differences between the two groups (fig. 4). Brain ECF lactate concentrations were constant during the 60 min of steady-state hypoxia and returned to the basal levels by the end of the experiment.

All animals that survived the hypoxic insult also survived for 24 h after the experiment. One day after the

<table>
<thead>
<tr>
<th>TABLE 1. Physiologic Variables in Hypoxic Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure (mmHg)</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Prehypoxia</td>
</tr>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Hypoxia, 90 min</td>
</tr>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Hypoxia, 90 min</td>
</tr>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Posthypoxia</td>
</tr>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6 for all data points.

* *P* < 0.05 versus prehypoxia values in each group.
experiment, spontaneous movements were rare and the animals' responses to stimulation were dull, although their extremities were not paralyzed. There were no obvious differences in behavior between the two groups.

SODIUM LACTATE INFUSION

The blood lactate concentration in the lactate-infused animals increased from 1.15 ± 0.22 mM to 9.54 ± 1.29 mM after 90 min of infusion, which was an 8.3-fold increase over the basal value. In contrast, brain ECF lactate concentrations increased from 1.19 ± 0.11 mM to 1.86 ± 0.09 mM, which was only a 1.5-fold increase over the basal value. Lactate concentrations, both in blood and brain ECF, decreased almost to the basal levels soon after the infusion ended (fig. 5). Figure 6 shows the relationship between the brain ECF and blood lactate concentrations 90 min after the beginning of the lactate infusion or after the onset of hypoxia in the saline- and glucose-infused groups. There were no significant differences in blood lactate concentrations between the lactate-infused group and the saline-infused hypoxic group or those between the lactate-infused group and the glucose-infused hypoxic group. However, the brain ECF lactate concentrations in both of the hypoxic groups were significantly higher than those in the lactate-infused group.

Discussion

The current data show that the brain ECF lactate concentration increased to 3.53 ± 0.20 mM from the basal level within 30 min after the induction of moderate hypoxia and remained constant during 60 min of steady-state hypoxia. It decreased to the basal level within 1 h after the end of hypoxia. These data also show that a high blood glucose concentration did not affect the brain ECF lactate concentration during moderate hypoxia. Gardinar et al. have shown a qualitatively similar reversible increase in brain tissue lactate concentrations (measured from frozen tissue extracts) during a shorter and more severe hypoxia (PaO2 of 20–22 mmHg for 15 min). The consistency of the results of the current study with theirs may confirm the reliability and usefulness of the microdialysis method in this model. With this method, the time course of changes in brain lactate concentrations and those of other variables during and after hypoxia could be measured simultaneously in living rats.

However, there are some quantitative differences between the current results and previously reported data. Brain tissue lactate concentrations during hypoxia of a degree similar to that used in the current study (PaO2 = 25–30 mmHg) were reported to be 7–10 μmol/g wet tissue (i.e., greater than the brain ECF lactate concentrations calculated in this study). As stated in the beginning of this article, we assumed that the brain intracellular and ECF lactate concentrations will equilibrate in a steady state, according to the data calculated by Kuhr et al. They showed that an increase in the brain intracellular lactate concentration was followed by an increase in the brain ECF lactate concentration and that the two equilibrated within several minutes after an electrocortical shock, as measured with an on-line microdialysis system in conscious rats. They suggested that when the brain adenosine triphosphate level is preserved, the brain cellular lactate transport is two times faster than the transport of lactate.

![Graph](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931325/)
across the BBB. Because the sampling interval in the current study was much longer than the delay of the change in the ECF lactate concentration, the calculated lactate concentration in the ECF was probably equal to the intracellular lactate concentration. If this is correct, it should be determined what caused the difference between the lactate concentrations as measured by microdialysis and those measured from tissue extracts.

It has been pointed out that there are several problems with both methods. A high lactate value tends to be obtained in tissue extracts because of postmortem metabolism, whereas a low value can be obtained by microdialysis in some tissues because the recovery rates of substances from the tissue interstitium through the probe membrane are lower than those from standard solutions. Future studies with more quantitative methods, such as in vivo calibration, may provide more accurate values.

The rate of lactate transport through the BBB is reported to be low (the Michaelis-Menten constant [Km] = 0.89 ± 0.25 [mM]; maximal velocity [Vmax] = 0.023 ± 0.006 [μmol · g⁻¹ · min⁻¹]), as shown in the sodium lactate infusion experiment, in which an increase in the blood lactate concentration as great as that observed during hypoxia caused little change in the brain ECF lactate concentration (fig. 5). Several studies also have shown that neither brain nor cerebrospinal fluid lactate concentrations increase after intravenous injection of large amounts of sodium lactate. It has been found that brain lactate concentrations are not changed by blood lactate concentrations during steady-state hypoxia, as shown in figures 3 and 4. Taken together, the data indicate that the increase in ECF lactate concentrations during steady-state hypoxia is produced mainly in brain cells, not derived from blood.

The low permeability of lactate through the BBB also blocks the flux of lactate from brain extracellular space to blood because the lactate transport system mediates the bidirectional flux across the BBB. Consequently, the lactate produced in the brain during hypoxia is thought to be used within the brain after hypoxia. Thus, the brain ECF lactate concentration would be determined mainly by two factors—the rate of lactate production and the rate of lactate utilization in brain cells. As shown in figure 4, the rates of lactate production and utilization reached a new equilibrium during steady-state hypoxia. Using hypoxic (Paco₂, approximately 30 mmHg) perfused isolated canine brain preparations, Kinnter et al. demonstrated that glucose utilization in the brain first increased and then gradually decreased as hypoxia continued; they suggested that this slowing of glucose utilization with continued hypoxia was modulated by the regulatory enzyme phosphofructokinase. As a result, the rate of lactate production in the tissue would be limited during steady-state hypoxia. Lactate utilization in the brain also may increase. Schurr et al. have shown that synaptic function in rat hippocampal slices continued to work normally in glucose-depleted artificial cerebrospinal fluid containing 2 mM or more of lactate. They suggested that lactate could be a superior energy substrate to glucose in the brain under hypoxic conditions. It seems likely that an increase in lactate production may accompany an increase in lactate utilization in the brain during moderate steady-state hypoxia, although the significance of lactate as an energy substrate in the brain is unknown.

The glucose supply in the brain is thought to depend solely on blood glucose concentrations because the glycogen store of the brain is small. Consequently, the rate of glucose transport through the BBB should be very high. The kinetic constants of glucose through the BBB are reported to be as follows: Km = 11.0 ± 1.4 (mM) and Vmax = 1.42 ± 0.14 (μmol · min⁻¹ · g⁻¹). It also has been reported that transport of glucose through the BBB does not limit cerebral glucose utilization during hypoxic (Paco₂ = 50 mmHg) perfusion in an isolated canine brain preparation. In the current study, however, there was a small but significant decrease in brain ECF glucose concentrations in saline-infused animals (fig. 2). This may mean that the glucose supply through the BBB can limit glucose utilization if anaerobic glycolysis is stimulated to a certain degree. However, the finding that glucose infusion did not accelerate the accumulation of lactate in brain ECF during hypoxia suggests that the glucose supply through the brain cell membrane was keeping up with the increase in glucose utilization in the brain cells even though the ECF glucose concentration was low. Gardina et al. also have shown that the brain tissue lactate concentration during hypoxia does not vary with the blood glucose concentration, and they suggested that glucose supply is not a rate-limiting factor for anaerobic glycolysis. However, with incomplete ischemia and significant hypoxia, the glucose load may cause a higher brain lactate concentration than that in the non–glucose-loaded animal.

Intracellular hydrogen ions are said to be responsible for modulation of cell injury caused by ischemia. In some studies, tissue lactate concentrations correlated directly with tissue acidosis during ischemia. However, others have suggested that the brain lactate concentration is not a valid marker of tissue acidosis. In the current experiment, although we observed some changes in the animals' behavior, the presence of direct neuronal injury was not tested. Therefore, in the future we must investigate the relationship between brain lactate accumulation and neuronal injury. In addition, in the current study rats were breathing spontaneously, and Paco₂ during steady-state hypoxia were low (17–19 mmHg), which should have increased the brain intracellular pH. Patients with acute respiratory failure commonly have much higher Paco₂.
which may cause more severe impairment than that expected from the results of the current study.

In conclusion, results from this study suggest that the glucose supply is not a rate-limiting factor for the amount of anaerobic glycolysis in the brain and, further, that lactate produced in brain tissue during hypoxia appears to be used within the brain during and after the period of hypoxia.

References


