Cerebral Blood Flow and Tissue Metabolism in Experimental Cerebral Ischemia of Spontaneously Hypertensive Rats with Hyper-, Normo-, and Hypoglycemia

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SUMMARY The present study was designed to clarify the effect of blood glucose level on cerebral blood flow and metabolism during and after acute cerebral ischemia induced by bilateral carotid ligation (BCL) in spontaneously hypertensive rats (SHR). Blood glucose levels were varied by intraperitoneal infusion of 50% of glucose (hyperglycemia), insulin with hypertonic saline (hypoglycemia) or hypertonic saline (normoglycemia). Cerebral blood flow (CBF) in the parietal cortex and thalamus was measured by hydrogen clearance technique, and the supratentorial metabolites of the brain frozen in situ were determined by the enzymatic method.

In non-ischemic animals, blood glucose levels had no influence on the supratentorial lactate, pyruvate or adenosine triphosphate (ATP) concentrations. In ischemic animals, however, cortical CBF was reduced to less than 1% of the resting value at 3 hours after BCL. However, there were no substantial differences of CBF during and after ischemia among 3 glycemic groups. Cerebral lactate in the ischemic brain greatly increased in hyperglycemia (34.97 ± 1.29 mmol/kg), moderately in normoglycemia (23.43 ± 3.13 mmol/kg) and less in hypoglycemia (7.20 ± 1.54 mmol/kg). In contrast, cerebral ATP decreased in hyperglycemia (0.93 ± 0.19 mmol/kg) as much as it did in normoglycemia (1.04 ± 0.25 mmol/kg), while ATP reduction was much greater in hypoglycemia (0.45 ± 0.05 mmol/kg). At 1-hour recirculation after 3-hour ischemia, ATP tended to increase in all groups of animals, indicating the recovery of energy metabolism. Such metabolic recovery after recirculation was good in hypoglycemia and normoglycemia, and was also evident in hyperglycemia.

Our results suggest that hyperglycemia is not necessarily an unfavorable condition in acute incomplete cerebral ischemia.

DURING THE LAST 25 YEARS, the diagnosis and therapy of cerebrovascular disease have improved, and the incidence and mortality of stroke have declined markedly. Early and effective treatment of hypertension might be one of the major causes in the reduction in the incidence of stroke. In recent years, however, epidemiologic studies indicate that diabetes mellitus or abnormal glucose tolerance is another important risk factor of ischemic stroke, although little is known about details of its mechanism and pathophysiology.

It has been reported that hyperglycemia has an adverse effect on energy metabolism in the complete ischemic brain because of severe lactic acidosis, and therefore, leads to poor clinical recovery from the insult. Our recent animal study, however, showed that brain energy metabolism in the incomplete 1-hour ischemia was less deranged in hyperglycemia than in normo- or hypoglycemia, even though ischemic metabolites such as lactate increased more in the former. However, we do not know how hyperglycemia affects brain tissue metabolism and blood flow in incomplete cerebral ischemia of longer than 1 hour. Therefore, the present study was designed to examine whether or not hyperglycemia is susceptible to cerebral ischemia by using an animal model established in our laboratory.

**Methods**

All of 101 female SHR, weighing 200–250 g, aged 5–9 months, were anesthetized with amobarbital (100 mg/kg body weight, i.p.). One femoral artery was cannulated to record the mean arterial pressure (MAP) and sample blood for determination of pH, pCO₂, pO₂, serum glucose concentration and hematocrit. Both common carotid arteries, exposed and separated carefully from the vasosympathetic trunks, were loosely encircled with sutures for later ligation and recirculation.

Thirty minutes prior to bilateral carotid ligation (BCL), hyperglycemia was produced by injection of 50% glucose solution (6 ml/kg, i.p.) and hypoglycemia was induced by injection of actrapid insulin (20 I.E/kg) with 8% NaCl (6 ml/kg, i.p.; osmotically equal to 50% glucose). Normoglycemic rats were prepared by infusion with 8% NaCl (6 ml/kg, i.p.). The animals in each group were divided into 3 subgroups; ischemia, recirculation and non-ischemic control. Brain ischemia was produced by bilateral ligation of common carotid arteries for 3 hours. Recirculation was made after 3-hour ischemia by release of the carotid ligation. In the control group, the carotid arteries were exposed but not ligated.

The animals breathed room air spontaneously and the rectal temperature was maintained close to 37°C.
throughout the experiment. Arterial blood samples were taken before and 1, 3 hours after BCL, and 1 hour after recirculation.

CBF Measurement

CBF in the cerebral cortex and thalamus was measured by the H₂ clearance method. The animal's head was fixed in a head-holder, and a small burr hole was made in the skull 2 mm lateral to the bregma on each side. A teflon-coated platinum electrode, 200 μm in diameter, with a 1 mm portion at its tip uncoated and plated with platinum black was placed in the parietal cortex (2 mm in depth from the brain surface) and another in the thalamus in the nucleus reticularis thalami (7 mm in depth) by using a stereotaxic apparatus. The reference electrode was an Ag-AgCl electrode inserted under the skin. H₂ clearance curves were obtained by the inhalation of 10% hydrogen gas. After allowing more than 30 min for a steady state, at least 3 base-line CBF measurements were made at intervals of about 10 minutes, then the carotid arteries were ligated. CBF was determined at 5 minutes and hourly up to 3 hours after BCL and 1 hour after recirculation.

Metabolism

At the end of the experiment, a plastic funnel was fitted into a skin incision over the skull bone and the head was frozen in situ by liquid nitrogen. The whole brain was chiselled out in the frozen state, and separated grossly into the supra- and infratentorial portions. In rapid sequence, the supratentorial part of the brain was weighed and ground after the addition of cold perchloric acid. The tissue homogenate maintained at 0-4 °C was centrifuged and neutralized with potassium hydrochloride at pH between 4.5 and 5.0. Lactate, pyruvate and ATP concentrations in the tissue homogenate were determined by standard enzymatic methods, as described previously.

Values were expressed as mean ± SEM and statistical differences were calculated by Student's small sample t-test.

Results

CBF measurement (fig. 1 and table 1)

Cortical CBF at rest did not differ from the blood glucose level. Following BCL, CBF decreased markedly to around 30% of the resting value at 5 minutes, further to about 10% at 1 hour and finally to an undetectably low level (less than 1%) at 3 hours. There were no differences in cortical CBF reduction during ischemia among 3 glycemic groups. One hour after recirculation, reduced CBF tended to increase in all animals, more greatly in hypoglycemic ones, but never reached the resting level (table 1).

Thalamic CBF similarly decreased close to 40-60% of the resting value immediately after BCL, and to 25% at 1 hour and 10% at 3 hours, followed by a small recovery during the reperfusion period. No significant differences were seen in the thalamic CBF during and after BCL among the 3 groups. The CBF reduction in the thalamus was less marked than that in the cortex.

MAP rose to 120% of the resting level immediately after BCL, followed by a gradual fall to 80% at 3 hours and further to 70% at 1 hour after recirculation. There were no essential differences in MAP, hematocrit and arterial acid-base parameters among the groups before, during and after ischemia. Arterial parameters at the end-point of experiments are shown in table 1.

Metabolism

Table 2 depicts mean values for serum glucose, and supratentorial brain lactate, pyruvate, lactate/pyruvate (L/P) ratio and ATP at the end-point of the study.

Serum glucose concentrations of non-ischemic control rats were 160 ± 12 mg/dl in normo-, 485 ± 70 mg/dl in hyper-, and 51 ± 17 mg/dl in hypoglycemia, respectively, all of these differences being significant. Blood glucose levels were also different among 3 glycemic groups at 3-hour ischemia as well as at 1-hour recirculation. During the period of both ischemia and recirculation in normo- or hyperglycemic animals, serum glucose levels increased significantly, probably due to acute cerebral ischemia induced secondary hyperglycemia (fig. 2).

Supratentorial lactate of non-ischemic control animals did not differ among 3 glycemic groups (fig. 3). During ischemia, however, lactate maximally increased in hyperglycemia (34.97 ± 1.29 mmol/kg), moderately in normoglycemia (23.43 ± 3.13 mmol/kg) and less markedly in hypoglycemia (7.20 ±
TABLE 1  Arterial Parameters and Cortical CBF in Normo-, Hyper- and Hypoglycemic Animals at the End-point of Experiment

<table>
<thead>
<tr>
<th>Condition</th>
<th>MAP (mm Hg)</th>
<th>pH</th>
<th>pCO₂ (mm Hg)</th>
<th>pO₂ (mm Hg)</th>
<th>Hct (%)</th>
<th>Cortical CBF (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ischemic control</td>
<td></td>
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<tr>
<td>normo (9)</td>
<td>180 ± 12</td>
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<tr>
<td>hyper (8)</td>
<td>167 ± 9</td>
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<tr>
<td>hypo (10)</td>
<td>178 ± 11</td>
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<td>Ischemia</td>
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<tr>
<td>normo (6)</td>
<td>155 ± 30</td>
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<tr>
<td>hyper (7)</td>
<td>140 ± 13</td>
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<tr>
<td>hypo (5)</td>
<td>143 ± 29</td>
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<td>Recirculation</td>
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<tr>
<td>normo (140</td>
<td>190 ± 9</td>
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<tr>
<td>hyper (131</td>
<td>282 ± 22</td>
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</tr>
<tr>
<td>hypo (147</td>
<td>299 ± 40</td>
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</table>

Values are mean ± SEM, (): number of rats, normo: normoglycemia, hyper: hyperglycemia, hypo: hypoglycemia. MAP: mean arterial pressure, Hct: hematocrit.

Statistical difference: vs normoglycemia.

Discussion

The brain is known to be the most susceptible organ to anoxia or even brief ischemia. It is also noted that the brain requires a great amount of high energy.
substrates for neuronal activity and neurotransmission. The main source for this requirement is glucose and oxygen from circulating blood. Physiologically glucose which is taken up in the brain tissue and is metabolized along the glycolytic pathway finally produces high energy phosphate, such as ATP. Changes in blood glucose concentrations, therefore, may affect brain metabolism, especially during and after cerebral ischemia.

Many previously reported findings in animals indicate that glucose administration prior to cerebral ischemia seriously aggravates the post-ischemic clinical outcome and impairs the restitution of metabolic activity.\textsuperscript{8-14} Siemcowicz\textsuperscript{12} found that all hyperglycemic rats died within 12 hours after 10 minutes of brain ischemia, while normoglycemic rats survived longer and recovered better from cerebral ischemia. It was evident that excessive lactate accumulated in the brain exposed to 15 to 30-minute ischemia in hyperglycemia and the recovery of both decreased cerebral ATP and phosphocreatine after 90-minute recirculation was severely impaired in the case of glucose pretreatment.\textsuperscript{13} These observations suggest that excess lactacidosis may be a major factor interfering with metabolic restitution following cerebral ischemia. Furthermore, in hyperglycemia there are many additional intra- and extracerebral factors causing unfavorable effects on brain metabolism such as attenuated cerebral oxygen utilization and blood flow, systemic metabolic acidosis, deranged hepatic metabolism, etc.\textsuperscript{10} The majority of these previous experiments, however, were achieved by using animal models of complete but not longer than 5 to 30 minutes ischemia of the brain. On the contrary, our model used in this study was incomplete but longer ischemia. The details of this model have been reported elsewhere from our laboratory.\textsuperscript{20, 21}

If blood glucose concentrations are reduced to below 18 mg/dl in animals, spontaneous EEG activity ceases and the cerebral energy state is extensively impaired even in the non-ischemic condition.\textsuperscript{22, 23} In contrast, our hypoglycemic animals of which the glucose level was much higher than 18 mg/dl, cerebral ATP remained normal at the control state. During 3-hour ischemia, however, ATP content decreased markedly to the level of less than one half of normoglycemia. Although cerebral lactate in such hypoglycemic-ischemic animals increased less than in normo- or hyperglycemic ones, a greater decrease in ATP suggests that hypoglycemic animals are more vulnerable to ischemic insult. Even moderate hypoglycemia of average 51 mg/dl, shown in this study, could possibly result in a rather severe disturbance of the brain energy metabolism during ischemia probably due to insufficient storage and supply of the substrate to the brain.

In hyperglycemic-ischemic animals, on the other
hand, lactate increased to the level beyond the so-called critical threshold of 25 mmol/kg, while ATP remained at almost the same level as that in normoglycemic-ischemic animals, suggesting that hyperglycemia per se seems unlikely to induce severe energy failure in the ischemic brain.

Following reperfusion, both the increased L/P ratio and decreased ATP were restored more greatly and quickly in the hypoglycemic animals. In contrast, in the hyperglycemic rats, pyruvate was rather slightly decreased while L/P ratio was increased after recirculation, suggesting that their 'metabolic machinery' — at least responsible for aerobic metabolism — is more severely damaged, and therefore, aerobic metabolism continues to be impaired in the hyperglycemic animals. According to our unpublished data, 8 of 11 hypoglycemic animals survived, while all of 9 normo- and 11 hyperglycemic ones were alive during 3 hours of ischemia. Following reperfusion, the survival rate reduced to 64% in hypog-, 78% in normo- and 91% in hyperglycemic rats at 1 hour, and to 46%, 56% and 64%, respectively at 20 hours. A longer survival in addition to less decreased ATP in hyperglycemic animals indicates that hyperglycemia during and after cerebral ischemia is not, at least, harmful to cerebral ischemia as many investigators have concluded.

At 5 hours after BCL, CBF was markedly reduced to less than 1% in the cortex and to 15% in the thalamus. This was a model of incomplete cerebral ischemia. CBF reduction during ischemia and CBF recovery after reperfusion showed no substantial differences among 3 glycemic groups except for a greater CBF recovery in hypoglycemia. Similarly, physiological parameters such as MAP, arterial acid-base balance and hematocrit did not differ among the groups before, during and after ischemia. Therefore, the metabolic changes found in this study could not be simply explained by the differences of hemodynamic alterations, but rather by the difference of blood glucose levels per se during and after cerebral ischemia.

In accordance with our results, Jernigan et al. very recently reported that both hyperglycemic and alloxan-induced diabetic rats showed lower morbidity and mortality scores than did normoglycemic rats after unilateral common carotid artery ligation with hypoxia. They concluded that hyperglycemia per se and in association with diabetes appeared to protect against anoxic/ischemic brain injury. There is also a report showing that pretreatment or concurrent administration of glucose leads to longer survival from anoxia in mice. It is likely if CBF is kept at a certain level, even very low during ischemia or anoxia, that hyperglycemia is desirable to produce ATP and to maintain cerebral metabolic activity. Long-term strict control of hyperglycemia in diabetics is undoubtedly able to prevent its vascular complications, but we must pay more attention to accidental or iatrogenic hypoglycemia in case of stroke which may cause more severe ischemic damage to the brain. Hyperglycemic vulnerability in cerebral ischemia has been emphasized in recent years, but our results and the results of others are against it. However, there are still more experimental and clinical problems to be solved in the future.

It is concluded from the present study that hyperglycemia is not necessarily harmful in acute cerebral ischemia and in the reperfused brain after ischemia. On the other hand, hypoglycemia should be avoided in cerebral ischemia, because it leads to severe metabolic disturbance of the brain due to an insufficient supply of the substrates to the ischemic brain.

References
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Laser Endarterectomy: A Comparison of Thrombotic Potential Following CO₂ Laser vs Surgical Endarterectomy


SUMMARY Although laser endarterectomy has recently been suggested as useful in the treatment of arteriosclerotic obstructions, the "in vivo" clotting effects have not been well delineated. In this study, the common carotid and femoral arteries of ten mongrel dogs were exposed, and alternating 1 cm segments of each artery were treated with surgical endarterectomy and low-powered CO₂ laser endarterectomy. Segments were then harvested, and subjected to histologic examination and vascular prostacyclin synthesis determinations, as measured by 6-keto-PGF₁α radioimmunoassay.

Gross examination and light and scanning electron microscopy showed increased platelet aggregation and more extensive damaging of the underlying media of the laser compared to the surgical segments. Six-keto-PGF₁α levels were significantly lower (p = 0.001) in the laser compared to surgical sites (mean 232 ± 72 pg/mg vs 515 ± 144 pg/mg), or controls (895 ± 337 pg/mg). These findings suggest that laser endothelial evaporation leads to increased thrombotic potential in the early post-operative period in comparison to surgical endarterectomy.

Laser endarterectomy, using a multiple portal co-axial catheter delivery system, has recently been proposed as a method to recanalize partially or totally occluded coronary arteries.¹⁻⁴ This technology also has great theoretical attractiveness in the treatment of arteriosclerotic cerebrovascular disease, as at least one-third of patients with carotid-distribution ischemic symptoms have obstructive lesions that are inaccessible to direct surgical correction (i.e. carotid endarterectomy).⁵

The specific effects of laser on vessel luminal surfaces have been largely limited to morphologic studies of small discrete (i.e. less than 1 mm diameter) lesions.² As most clinically significant carotid or vertebral-basilar stenoses represent considerably greater luminal surface area, the direct application of these results in the treatment of cerebrovascular lesions may not be appropriate. Furthermore, the alterations of the clotting mechanism induced by intimal and media exposure to laser energy have not been well delineated. Since many untoward events in the cerebrovascular circulation are related to clotting abnormalities (i.e. thrombosis and embolism), the laser-induced effects on these mechanisms must be thoroughly analyzed before application to cerebral lesions can be safely considered.

In this study, the effects of low power CO₂ laser intimal and partial medial vaporization (herein termed laser endarterectomy) were investigated, both by light and scanning electron microscopy and by prostacyclin biosynthesis. These results were then compared to those changes induced by surgical endarterectomy, to evaluate the differences in clotting alterations induced by both treatments.

Materials and Methods

Ten mongrel dogs (15–25 kg) were anesthetized with intravenous pentobarbital (35 mg/kg), intubated, and placed on a large animal ventilator (Harvard venti-
Cerebral blood flow and tissue metabolism in experimental cerebral ischemia of spontaneously hypertensive rats with hyper-, normo-, and hypoglycemia.
S Ibayashi, M Fujishima, S Sadoshima, F Yoshida, O Shiokawa, J Ogata and T Omae

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