Moderate Hyperglycemia Worsens Acute Blood–Brain Barrier Injury After Forebrain Ischemia in Rats

W. Dalton Dietrich, PhD; Ofelia Alonso, BS; and Raul Busto, BS

**Background and Purpose:** Clinical and experimental data indicate that hyperglycemia can aggravate the consequences of stroke and cerebral ischemia. The purpose of this study was to examine the effects of moderate hyperglycemia on the response of the blood–brain barrier to normothermic (37°C) and hypothermic (30°C) global forebrain ischemia.

**Methods:** Sixteen rats underwent 20 minutes of four-vessel occlusion followed by 30 minutes of postischemic recirculation. We used the protein tracer horseradish peroxidase as an indicator of increased vascular permeability, and rats were perfusion-fixed for microscopic analysis. To produce moderate hyperglycemia, we gave an intraperitoneal injection of 50% dextrose 15 minutes before the ischemic insult.

**Results:** After normothermic brain ischemia, normoglycemic rats (plasma glucose level, 115±3 mg/dl) demonstrated extravasated horseradish peroxidase mainly restricted to the cerebral cortex. In contrast, more severe and widespread protein extravasation was documented throughout the neuraxis of hyperglycemic (plasma glucose level, 342±27) rats. Sites of protein leakage included the cerebral cortex, striatum, hippocampus, thalamus, and cerebellum. Foci of protein extravasation were associated with pial and large penetrating vessels. Intraischemic hypothermia significantly attenuated the blood–brain barrier consequences of hyperglycemic brain ischemia.

**Conclusions:** Under normothermic ischemic conditions, hyperglycemia significantly worsens the degree of acute blood–brain barrier breakdown compared with normoglycemia. Postischemic blood–brain barrier disruption may play an important role in the pathogenesis of increased brain damage associated with systemic hyperglycemia. *(Stroke 1993;24:111–116)*

**KEY WORDS** • blood–brain barrier • cerebral ischemia • hyperglycemia • temperature • rats

Hyperglycemia has been shown to worsen ischemic outcome in various models of global and focal cerebral ischemia.1–7 Myers and Yamaguchi8 first reported that monkeys given glucose before cardiac arrest developed greater neurological disturbances and more severe histopathological injury compared with normoglycemic animals. In another study, Siemkowicz and Hansen2 showed that normoglycemic rats survived chronically after 10 minutes of complete ischemia, but hyperglycemic rats died within 24 hours. In a model of forebrain ischemia, Pulsinelli and colleagues5 reported that rats given glucose before a 20-minute ischemic insult demonstrated augmented morphological damage and brain edema. Enhanced brain damage after hyperglycemic cerebral ischemia is believed to be a consequence of increased lactate production leading to neuronal or astrocytic acidosis.8,9

Clinical findings indicate that elevated levels of blood glucose are a risk factor for stroke and are associated with a worse prognosis.10–12 In a retrospective review of 39 stroke patients, Berger and Hakim13 concluded that hyperglycemic patients with serum glucose values greater than 150 mg/dl developed more pronounced cerebral edema and had a worse clinical outcome compared with patients with glucose values less than 100 mg/dl. Increased brain edema after hyperglycemic brain ischemia might be a consequence of aggravated postischemic blood–brain barrier (BBB) disruption. However, a previous study by Siemkowicz13 failed to demonstrate disturbances in BBB function after 10 minutes of global ischemia.

Recent experimental studies have demonstrated the importance of ischemic brain temperature on the development of postischemic vascular damage and BBB disruption.14,15 Although mild intraischemic hyperthermia (33°C) significantly attenuates BBB damage after global ischemia, intraischemic hyperthermia (39°C) aggravates barrier disruption compared with normothermia (36°C). The BBB consequences of hyperglycemic
brain ischemia have not been studied under temperature-controlled ischemic conditions.

The purpose of this study was to determine whether preischemic hyperglycemia exaggerates the BBB consequences of normothermic cerebral ischemia. We also determined whether intraischemic hyperthermia, previously shown to reduce neuronal injury under both normoglycemic and hyperglycemic conditions,\textsuperscript{16,17} attenuates the BBB consequences of hyperglycemic brain ischemia.

Materials and Methods

Brain Ischemia

Sixteen male Wistar rats weighing 250–300 g were subjected to 20 minutes of severe forebrain ischemia induced by four-vessel occlusion combined with systemic hypotension.\textsuperscript{16} One day before the ischemic insult, both vertebral arteries were electrocoagulated with rats under anesthesia of 3% halothane and a mixture of 70% nitrous oxide and 30% oxygen. The next day, the femoral vessels were cannulated with rats under halothane anesthesia, and polyethylene ligatures were placed around each common carotid artery. Rats then were intubated and mechanically ventilated to maintain PaCO\textsubscript{2} and PaO\textsubscript{2}, within normal ranges. After a 1-hour stabilization period, 20 minutes of severe incomplete ischemia was induced by tightening the carotid ligatures bilaterally and maintaining mean arterial blood pressure at 80 mm Hg by gradual withdrawal of blood. In normothermic rats, brain and body temperatures were maintained at 37°C by a thermostatically regulated heating lamp. Pericranial temperature was monitored with a probe placed in the temporalis muscle. To produce intraischemic brain hypothermia, we blew cool air onto the rat’s head during the ischemic insult.

Hyperglycemic rats (n=11) received an intraperitoneal injection of 1.5 ml of 50% dextrose 15 minutes before the ischemic insult. This procedure results in plasma glucose levels that have been reported to worsen the neurological and histopathological consequences of incomplete cerebral ischemia.\textsuperscript{5,18} In addition, this method of increasing blood glucose level does not significantly alter serum osmolality.\textsuperscript{19} Normoglycemic rats (n=5) were fasted overnight and received a comparable volume of saline.

Morphological Study

After the 20-minute ischemic insult, carotid ligatures were removed and cerebral recirculation was initiated. Blood kept at 37°C was reinfused to restore arterial blood pressure to 100–120 mm Hg. At 15 minutes after the start of recirculation, 30 mg/ml horseradish peroxidase (HRP, type II, Sigma Chemical Co., St. Louis, Mo.) dissolved in 1 ml saline was injected intravenously over a 2-minute period. Thirty minutes after the start of recirculation, rats were perfused transcardially with 0.9% saline followed by 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M sodium phosphate buffer. Brains were then processed for the microscopic visualization of HRP by methods previously described.\textsuperscript{14}

To determine the relative density of leaky vessels per hemisphere, we counted the permeable sites in dry-mounted Vibratome sections of individual brain regions. At the level of the anterior commissure, numbers of leaky vessels were determined in cortical and striatal areas. At the level of the anterior hippocampus, the number of leaky vessels was also determined in the parietal cortex, thalamus, and hippocampus. Finally, leaky sites were counted in the cerebellar cortex and medulla. BBB alterations were then grouped according to a semiquantitative scale: 0 = no leakage in a given brain region; 1 = few leaky vessels (less than three); 2 = many leaky vessels (greater than three). Physiological data were compared by one-way analysis of variance. The Scheffe’s and Dunn procedures were used to correct for multiple comparisons.\textsuperscript{20} Semiquantitative BBB data were compared using the Kruskal-Wallis one-way analysis of variance by ranks.

Results

Physiological Variables

Table 1 summarizes the physiological findings in preischemic rats. Blood glucose values were significantly elevated in both normothermic and hyperthermic hyperglycemic rats compared with saline-treated rats. No significant differences in any of the other physiological variables were noted between the experimental groups.

Normoglycemic Rats

In normothermic ischemic rats given saline, extravasated HRP was seen throughout the cerebral cortices of four of five rats (Figure 1A, Table 2). Symmetrical patches of extravasated HRP were commonly columnar in orientation and associated with penetrating vessels. Extravasated HRP was also observed within surrounding neuronal cell bodies and processes. Mild striatal and hippocampal leakage was seen in five hemispheres. In the striatum, extravasated HRP was detected within the dorsolateral portion of that structure. In two of five rats, unilateral BBB breakdown was also seen in the thalamus. In these cases, leakage was mild and restricted to the ventrolateral thalamic nucleus. In other brain regions, extravasated HRP was not seen.

Hyperglycemia

In hyperglycemic rats that underwent normothermic brain ischemia, HRP extravasation was more widespread and severe than that observed in rats under normoglycemic conditions (Figures 1B–1F, 2A, and 2B; Table 2). In this experimental group, extravasated HRP was detected throughout the cerebral cortex, striatum, septum, hippocampus, thalamus, and cerebellum. In the cingulate cortex, protein extravasation was intense and frequently involved the corpus callosum (Figure 2A).

Table 1. Physiological Variables in Preischemic Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normoglycemia, 37°C (n=5)</th>
<th>Hyperglycemia, 37°C (n=6)</th>
<th>Hyperglycemia, 30°C (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paco\textsubscript{2} (mm Hg)</td>
<td>40±3</td>
<td>43±3</td>
<td>38±2</td>
</tr>
<tr>
<td>PaO\textsubscript{2} (mm Hg)</td>
<td>129±9</td>
<td>145±11</td>
<td>127±9</td>
</tr>
<tr>
<td>pH</td>
<td>7.43±0.03</td>
<td>7.36±0.025</td>
<td>7.46±0.03</td>
</tr>
<tr>
<td>MAPB (mm Hg)</td>
<td>127±10</td>
<td>123±6</td>
<td>123±5</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>115±3</td>
<td>342±27*</td>
<td>288±21*</td>
</tr>
</tbody>
</table>

Values are mean±SEM recorded immediately before ischemic insult. MAPB, mean arterial blood pressure.

*Significantly different from normoglycemic rats.
Within the somatosensory cortex, extravasated HRP was associated with penetrating vessels. Pial vessels also demonstrated extravasated HRP, with brain surfaces being outlined by reaction product. In the striatum, extravasated HRP was pronounced in the dorsolateral sector and occasionally associated with the lenticulostriate artery. Within the hippocampus, leaky vessels running within the hippocampal fissure were also commonly observed (Figures 1C, 1E, and 2B). Focal sites of thalamic BBB disruption were detected in all five hyperglycemic rats and involved the ventrolateral nucleus and lateral reticular nucleus. Extravasated HRP within the cerebellar cortex (Figure 1F) was detected in three of five rats, whereas focal leakage within the brain stem was seen in two of five rats.

**Hypothermia**

Intraischemic hypothermia (30°C) significantly attenuated the BBB consequences of hyperglycemic brain ischemia (Table 2, Figures 2C and 2D). In three of five rats, sites of dense extravasated HRP were not apparent. In the two rats that demonstrated extravasated HRP, the reaction product was both mild and diffuse.
Table 2. Semiquantitative Assessment of Blood–Brain Barrier Alterations

<table>
<thead>
<tr>
<th>Ischemic group</th>
<th>BBB grade</th>
<th>% Hemispheres with grades 0–2</th>
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<tbody>
<tr>
<td></td>
<td>Cerebral cortex</td>
<td>Striatum</td>
</tr>
<tr>
<td>Normoglycemia, 37°C (n=10)</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td>Hyperglycemia, 37°C (n=12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Hyperglycemia, 30°C (n=10)</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td></td>
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<td>50</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
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Significance

<table>
<thead>
<tr>
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<th>P</th>
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<tbody>
<tr>
<td>0.13</td>
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</tr>
<tr>
<td>13.83</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>22.42</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>22.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>14.60</td>
<td>&lt;0.01</td>
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<tr>
<td>0.43</td>
<td>NS</td>
</tr>
</tbody>
</table>

Blood–brain barrier (BBB) grades: 0=No leakage of horseradish peroxidase; 1=few leaky vessels (<3); 2=many leaky vessels (>3). Significance assessed by Kruskal-Wallis one-way analysis of variance. NS, not significant.

Discussion

The present investigation demonstrated that moderate hyperglycemia significantly aggravated the BBB consequences of transient forebrain ischemia. It is important to emphasize that these permeability findings were documented during the acute phase of posts ischemic recirculation. These findings therefore indicate that hypoglycemia may potentially worsen ischemic brain injury by increasing cerebrovascular permeability.

In early studies by Myers,21 glucose-infused monkeys subjected to 10 minutes of cardiac arrest were reported to demonstrate widespread BBB breakdown to Evans blue. However, using a rat model of transient global ischemia, Siemkowiez13 failed to show a significant effect of preischemic hyperglycemia on the BBB. A possible explanation for these inconsistent findings is derived from recent data showing that ischemic brain temperature is a critical factor in determining the severity of posts ischemic BBB injury.14,15 In one study in which intraischemic brain temperature was artificially varied from 30–39°C during a 20-minute ischemic insult, mild hypothermia (33°C) was shown to inhibit the

![Figure 2](http://stroke.ahajournals.org/) Panel A: Photomicrograph shows protein extravasation within anterior cingulate cortex from hyperglycemic rat (plasma glucose level, 253 mg/dl). Permeable vessels are also seen within the underlying corpus callosum. Panel B: Photomicrograph shows leaky blood vessels within hippocampal fissure of hyperglycemic rat (plasma glucose level, 345 mg/dl). Panel C: 30°C, Hyperglycemia (blood glucose level, 314 mg/dl). Photomicrograph shows diffuse extravasated protein primarily within right dorsolateral striatum. Panel D: 30°C, Hyperglycemia (blood glucose level, 298 mg/dl). Photomicrograph shows mild protein leakage in thalamus.
BBB changes seen under normothermic (36°C) ischemic conditions. Because intraschismic brain temperature spontaneously falls in anesthetized rats during global ischemia, different intraschismic brain temperatures may be one explanation for inconsistent findings regarding hyperglycemic brain ischemia and the BBB. Hyperglycemia has been shown in some ischemia models to accentuate postischemic brain edema. In one study, preischemic hyperglycemia significantly increased brain water in rat forebrain structures 24 hours after global ischemia compared with normoglycemic rats. In another study, although both normoglycemic and hyperglycemic rats displayed an increase in brain water at 1.5 hours after 10 minutes of forebrain ischemia, the resolution of brain edema was slower and more variable in the hyperglycemic rats. In that study, although the initial increase in brain water was fully resolved by 12 hours in hyperglycemic rats, a secondary increase in water was reported at 24 hours. Although some studies have demonstrated a close correlation between brain edema and the extravasation of protein tracers, regional measurements of brain water are required to determine whether the present BBB alterations are associated with increased edema formation. The detrimental effects of preischemic hyperglycemia on cerebral blood vessels and postischismic cerebral blood flow have been documented previously. Paljarvi and colleagues reported that glucose pretreatment caused vascular endothelial swelling and luminal narrowing after 30 minutes of global ischemia in rats. It is not known how hyperglycemia may affect endothelial structure or worsen postischismic BBB breakdown. Because immediate postischemic BBB opening may be the result of reactive hyperemia, hyperglycemia and subsequent tissue lactate acidosis may affect the magnitude of the hyperemic phase. This hypothesis is consistent with published data indicating that the duration and regional patterns of postischismic hyperemia vary significantly after complete and incomplete ischemia. Preischismic hyperglycemia has been shown to enhance neuronal and glial injury after global forebrain ischemia. Thus, the present microvascular findings could also be a secondary response to neuronal or astrocytic damage. Injured neurons may release permeability-inducing substances, including platelet activating factor, serotonin, or possibly free radicals, which could promote BBB disruption. Because astrocytes are involved in the maintenance of the BBB, injury to this cell type under hyperglycemic conditions might also lead to permeability alterations. The mechanism by which hyperglycemia affects postischismic BBB disruption may be multifactorial, possibly involving several vasoactive substances as well as multiple cell types. In summary, hyperglycemic brain ischemia carried out under normothermic conditions led to acute postischismic BBB damage. Intraischemic hypothermia significantly attenuated the BBB changes. In addition to neurons and astrocytes, hyperglycemic ischemia severely affects cerebrovascular endothelial integrity. Increased postischismic vascular permeability may represent an important pathomechanism by which hyperglycemia worsens ischemic outcome.

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**References**

This study represents an extension of previously published work from this laboratory with respect to the effects of transient forebrain ischemia (four- vessel occlusion model) and the influence of intrasichemic temperature on horseradish peroxidase extravasation during the acute recirculation period. The new findings presented in this report are that hyperglycemia worsens the blood–brain barrier (BBB) breakdown and that modest intrasichemic hypothermia can attenuate this effect of hyperglycemia. The extravasation of a plasma protein marker could be taken as evidence that this model gives rise to a vasogenic edema in the immediate postischemic period, which in turn may contribute to a worsened outcome. The major implications of this work are the following: 1) the worsened outcomes associated with intrasichemic hyperglycemia described in many earlier studies may have a BBB breakdown and/or cerebral edema component; and 2) modest hypothermia, as well as protecting neurons, can "protect" the BBB from the damaging influences of hyperglycemia. This simple, descriptive study does not provide any clues as to the cause for the observed BBB changes, including whether the changes are the result of a primary action at the cerebral endothelium or occur secondarily in association with neuronal/glial damage. Nevertheless, the authors do provide some speculation in this regard. Their suggestion that "permeability-inducing" substances (e.g., platelet activating factor, free radicals) may be released from damaged brain cells is particularly intriguing and could provide some impetus for future investigation.

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References


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