Hyperglycemia Decreases Acute Neuronal Ischemic Changes After Middle Cerebral Artery Occlusion in Cats

Milford A. Zasslow, MD, Ronald G. Pearl, MD, PhD, Lawrence M. Shuer, MD, Gary K. Steinberg, MD, PhD, Robert E. Lieberson, MD, and C. Philip Larson Jr., MD

Hyperglycemia has been reported to worsen the tolerance of the brain to ischemia, and it has therefore been recommended that patients undergoing neurosurgical procedures not receive glucose-containing solutions. However, whereas most animal studies have used global ischemia models, most neurosurgical procedures are associated with risks of focal rather than global ischemia. We therefore studied the effects of glucose administration in an animal model of focal cerebral ischemia. We anesthetized 20 cats with halothane (0.85% end tidal in oxygen), and a focal cerebral ischemic lesion was produced by clip ligation of the left middle cerebral artery using a transorbital approach. Hyperglycemia (10 cats, mean±SEM plasma glucose concentration 561 ±36 mg/dl) was established before ligation by infusion of 50% glucose in 0.45% saline; the control group (10 cats, mean±SEM plasma glucose concentration 209 ±28 mg/dl) received 0.45% saline only. Total fluid administered, mean arterial blood pressure, body temperature, and arterial blood gas values did not differ between the two groups 0, 2, and 6 hours after ligation. The cats were killed 6 hours after ligation, and the area of severe ischemic neuronal damage was determined by microscopic examination of a coronal section at the level of the optic chiasm. The mean±SEM area of left cortical severe ischemic neuronal damage was 12±2% of the left cortex in the hyperglycemic group compared with 28±5% in the control group (p<0.01). The area of severe ischemic neurologic damage was inversely related to plasma glucose concentration, for both all cats (r=0.69) and for the control cats alone (r=0.80). We conclude that glucose may protect the brain from severe, focal, ischemic neuronal damage and that further studies are needed to document the efficacy and mechanisms of protection. (Stroke 1989;20:519–523)

In models of global cerebral ischemia, hyperglycemia increases both morphologic brain damage1-3 and the severity of neurologic deficits.4,5 In these models, temporary global cerebral ischemia is produced by a variety of techniques (including hypoxia, hemorrhage, tourniquet interruption of cerebral blood flow, surgical occlusion of the cerebral vessels, induced hypotension, and cardiac arrest), often in combination, followed by restoration of normal cerebral oxygenation and circulation. However, the generalizability of these models to clinical practice is debatable since cerebral ischemia in humans is often focal rather than global. Interventions may have different effects in focal compared with global cerebral ischemia. For example, the administration of barbiturates before ischemia appears to provide protection from focal cerebral ischemia6,7 but is ineffective in global ischemia.8 The effect of hyperglycemia on focal cerebral ischemia remains controversial. Several studies suggest that hyperglycemia worsens the injury,9-11 whereas others suggest that it either decreases12,13 or has no effect on14 the extent of cerebral injury. To evaluate this question, we examined the effects of hyperglycemia in a cat model of permanent focal cerebral ischemia.

Materials and Methods

We studied 20 cats of either sex, weighing 2–4 kg. The study was approved by the Institutional Panel.
on Laboratory Animal Care. The cats were fed until 24 hours before the experiment; subsequently they received only water. On the day of study, anesthesia was induced with 10 mg/kg i.v. ketamine. Following tracheal intubation, the lungs were mechanically ventilated with a Harvard ventilator (South Natick, Massachusetts) adjusted to maintain PaCO₂ between 36 and 40 mm Hg. Anesthesia was maintained with halothane in oxygen at an end-tidal concentration of 0.85% (Puritan-Bennett anesthetic agent monitor, model 222, Westmont, Illinois). End-tidal CO₂ was measured with an infrared gas analyzer (Puritan-Bennett CO₂ monitor). A femoral artery and vein were cannulated by cutdown. Mean arterial blood pressure (MABP) was monitored continuously (Hewlett-Packard quartz transducers and four-channel monitor, Palo Alto, California) and maintained between 80 and 120 mm Hg using saline infusion as needed. Arterial blood gases were measured (Corning 168 pH/blood gas analyzer, Corning, New York) after the institution of mechanical ventilation, immediately before clipping of the middle cerebral artery (MCA), and 1, 2, and 6 hours after MCA clipping. Arterial blood gas data at 1 hour was the same as that at 2 hours and therefore is not reported. Rectal temperature was maintained at 36.5–38°C with warming blankets.

The cats were randomized into two groups. Following femoral vein cannulation, both groups received a fluid infusion of 8 ml/kg over 5 minutes followed by continuous infusion at 8 ml/kg/hr. In the glucose group, the fluid was 50% glucose in 0.45% saline; in the control group, the fluid was 0.45% saline.

Focal cerebral ischemia was produced by clipping the left MCA using the transorbital approach described by O'Brien and Waltz. The clip was applied approximately 45 minutes after the start of fluid infusion. Blood for the determination of arterial blood gas values and plasma glucose concentration was obtained before MCA clipping and 2 and 6 hours later.

Six hours after MCA clipping, a sternotomy was performed. The descending aorta was clamped, the right atrium was opened to drainage, and 300 ml 0.9% saline followed by 300 ml 10% buffered formalin was infused into the aortic root. The brain was removed and fixed for 2 weeks in formalin, after which 10-μm coronal, paraaffin-embedded sections were made and stained with hematoxylin and eosin. A microscopic section through the optic chiasm was chosen for histologic evaluation.

Using a previously described grading system (Table 1), ischemic neuronal changes were assessed without knowledge of the treatment group. Grade 2 and 3 changes were considered to represent severe ischemic neuronal damage (SIND). The area of SIND was marked on an 8½ x 11 cm photographic reproduction of each slide. The areas of cortical SIND and total left cortex in the section were measured by computerized planimetry using a digitizing tablet (Summagraphics, Fairfield, Connecticut) and auto-CAD software (Autodesk, Sausalito, California). The area of SIND was expressed as a percentage of the area of the left hemispheric cortex. Values for the groups are expressed as mean±standard error of the mean (SEM).

Areas of SIND were compared between groups using Student's t test and the Mann-Whitney U test. MABP, temperature, arterial blood gas values, and plasma glucose concentration were analyzed by two-factor repeated-measures analysis of variance and the Newman-Keuls test. Plasma glucose concentration and SIND were correlated using standard linear regression analysis; p<0.05 was considered significant.

**Results**

There were no significant differences between groups with respect to the volume of fluid administered during the study or to the values for MABP, temperature, or arterial blood gases 0, 1, 2, and 6 hours after MCA clipping (Table 2). Plasma glucose concentrations were significantly higher in the glucose than the control group at all three times (Table 2) but were relatively constant within each group throughout the study.

The area of SIND ranged from 6% to 58% of the left cortex (Figure 1), with the mean±SEM being 12±2% in the glucose group and 28±5% in the control group (p<0.01 by t test and Mann-Whitney U test). The area of SIND was inversely correlated with the plasma glucose concentration among all cats at the time of MCA clipping (r=0.69, p<0.01; Figure 2), 2 hours after MCA clipping (r=0.58, p<0.01), and 6 hours after MCA clipping (r=0.58, p<0.01). The inverse correlation was also present in the control group alone at the time of MCA clipping (r=0.80, p<0.01; Figure 3), 2 hours after MCA clipping (r=0.82, p<0.01), and 6 hours after MCA clipping (r=0.54, p<0.05).

**Discussion**

In our model of focal cerebral ischemia, there was an inverse relation between the blood glucose concentration and the area of SIND in the left cortex. Even in the absence of glucose administration, endogenous variations in plasma glucose concentration were important to outcome, as evi-

**Table 1. Grades of Neuronal Ischemia According to the Method of Little**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Morphologic description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Slight shrinkage, loss of Nissl substance, and/or cytoplasmic vacuolation</td>
</tr>
<tr>
<td>2</td>
<td>Moderate shrinkage, cytoplasmic eosinophilia, and increased nuclear basophilia or moderate swelling; pale, vacuolated cytoplasm; and distended, vesicular nucleus</td>
</tr>
<tr>
<td>3</td>
<td>Severe shrinkage, bright cytoplasmic eosinophilia, pyknotic nucleus, and incrustations</td>
</tr>
</tbody>
</table>
TABLE 2. Physiologic Data for Cats Subjected to Focal Cerebral Ischemia

<table>
<thead>
<tr>
<th>Time</th>
<th>Control group</th>
<th>Glucose group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>0</td>
<td>107±8</td>
<td>36.8±0.3</td>
</tr>
<tr>
<td>2</td>
<td>99±7</td>
<td>36.6±0.3</td>
</tr>
<tr>
<td>6</td>
<td>94±6</td>
<td>36.8±0.5</td>
</tr>
</tbody>
</table>

Data are mean±SEM, 10 cats per group. Time after clipping of middle cerebral artery.
*p<0.01 different from control.

The presence of hyperglycemia was evident by the significant, inverse relation for the control group (Figure 3). Although we did not measure glucose concentrations in the brain, it is reasonable to assume that they were much higher than normal in the glucose group since glucose transport across the blood–brain barrier is directly related to blood glucose concentration. In a study in rats by Ginsberg et al., a threefold increase in plasma glucose concentration produced a threefold increase in brain glucose concentration.

Our observation that glucose lessens the morphologic changes associated with unilateral ischemia is in agreement with the findings in rats by Ginsberg et al. and Jernigan et al., but not with others in which focal transient or permanent occlusion were used, or with the several studies in which global ischemia was used. It is difficult to compare our results with those of other studies because the protocols are distinctly different. For example, in the study in rats by Ginsberg et al., focal cerebral ischemia was produced by photochemically inducing a unilateral thrombotic infarction, which required many hours to develop; in contrast, in our study focal ischemia was immediate. In the study of Ginsberg et al., the rats survived 7 days before the brains were removed for histologic analysis of the extent of ischemic injury, whereas in our study the cats were killed 6 hours after MCA clipping.

In a study in cats by Venables et al., hyperglycemia was induced after a period of cerebral ischemia, not before or during the ischemic episode. In that study, MCA occlusion was maintained for 2 hours, during which time no exogenous glucose was administered; the clip was then removed and the brain was reperfused for 1 hour with fluid containing either normal saline or 10% glucose. Local cortical

![FIGURE 1. Bar graph, area of severe ischemic neuronal damage (SIND) (Grades 2 and 3 according to Little) expressed as percent of left cortex in 20 cats. Ten cats received infusion of 50% glucose in 0.45% saline (open bars), which produced mean±SEM plasma glucose concentration of 561±36 mg/dl before clipping of middle cerebral artery. The other 10 cats (control group, hatched bars) received 0.45% saline and had mean±SEM plasma glucose concentration of 209±28 mg/dl before clipping. Area of SIND was greater in control cats (p<0.01 by t test and Mann-Whitney U test).](http://stroke.ahajournals.org/)

![FIGURE 2. Relation of area of severe ischemic neuronal damage (SIND) (Grades 2 and 3 according to Little) expressed as percent of left cortex to plasma glucose concentration at time of clipping of middle cerebral artery in 20 cats. Ten cats received 0.45% saline (control group, filled squares) and 10 received 50% glucose in 0.45% saline (open squares) (r=0.69, p<0.01).](http://stroke.ahajournals.org/)
blood flow, cortical specific gravity, γ-aminobutyric acid uptake, and pial surface potassium activity were adversely affected during reperfusion in the hyperglycemic cats. Clearly, this model examines only the effects of hyperglycemia induced after reperfusion, whereas our model examines the effects of hyperglycemia induced before and maintained during 6 hours of focal cerebral ischemia without reperfusion to the ischemic area.

In a study of cerebral ischemia in rats made hyperglycemic by injection of streptozotocin 2 days before MCA clipping, Nedergaard found that hyperglycemia resulted in increased cerebral infarction in a model of transient focal ischemia but not in one in which the occlusion was permanent. Furthermore, in the permanent focal ischemia model, hyperglycemia appeared to decrease the extent of selective neuronal damage adjacent to the infarct compared with the effects of acute hypoglycemia induced by insulin given 2 hours before MCA occlusion. These findings suggest that the effects of hyperglycemia may differ under conditions of transient versus permanent cerebral ischemia due to the influence of reperfusion after temporary occlusion.

The global ischemia models are consistent in that they uniformly demonstrate that hyperglycemia worsens tolerance to cerebral ischemia. However, it is well recognized that drugs may have distinctly different effects in global and focal cerebral ischemia. For example, barbiturates appear to protect the brain from focal, but not global, ischemia. This difference is most likely related to the ability of barbiturates to preferentially divert blood flow to focally, but not globally, ischemic brain. Barbiturates are potent cerebral vasoconstrictors, and in the presence of focal ischemia barbiturates constrict the normally reactive cerebral vessels, thereby enhancing blood flow to the ischemic area.

In contrast, if all cerebral vessels are dilated because of global ischemia, no redistribution of blood flow occurs.

It is uncertain why hyperglycemia worsens neurologic injury in global ischemia but appears to have protective effects in focal ischemia. In global ischemia, high concentrations of glucose in brain tissue may enhance anaerobic metabolism above that which would occur if less glucose were present, thereby increasing the quantities of lactic acid formed. The excess hydrogen ions liberated by the dissociation of lactic acid are believed to be highly toxic to brain tissue, causing a cascade of destructive changes including increased influx of sodium and calcium into neuronal cells, lipolysis, proteolysis, membrane damage, and cellular catabolism. If the ischemia is more focal, not only may the magnitude of anaerobic metabolism be insufficient to cause appreciable hydrogen ion formation or accumulation, but the additional glucose may also enable the border zones of ischemia to maintain viability. There is some evidence that glucose in the absence of lactic acid facilitates recovery from injury. Schurr and colleagues found that perfusing rat hippocampal slices with a hyperglycemic solution facilitated recovery of synaptic function following exposure to hypoxia. Presumably, in the absence of excess hydrogen ions, the increased substrate availability provided by glucose lessens injury induced by oxygen deprivation. The extent of collateral circulation may also affect the cerebral ischemic response to hyperglycemia. Prado et al recently reported a study in rats; they observed that regions of brain in which the collateral circulation is extensive are highly susceptible to the toxic effects of hyperglycemia, whereas regions having a nonanastomosing circulation are not.

There are several limitations to our study that must be considered when interpreting our findings. First, we killed our cats 6 hours after MCA clipping; therefore, we cannot draw any conclusions about functional impairment or ultimate histologic changes. However, studies by both one of us and by others have shown that distinct histopathologic changes of severe ischemic neuronal damage are evident by 6 hours after MCA occlusion in cats. We also cannot draw any conclusions about the effects of hyperglycemia on noncortical brain tissue since this was not examined. Second, it is possible that the beneficial effect of hyperglycemia in our study may be due not to glucose per se but rather to a hyperosmotic effect causing, for example, an improvement in the microcirculation as has been reported with other hyperosmotic agents such as mannitol and dextran. However, Ginsberg et al reported that an osmotic load of mannitol comparable to the glucose load we used increased infarct volume in rats subjected to unilateral thrombotic infarction compared with that observed in saline-treated control rats.
Based on global ischemia studies, it has been recommended that glucose-containing solutions be avoided in patients undergoing neurosurgical procedures. We believe that there are insufficient data at present to make any recommendations about the use of glucose during neurosurgery. Our study and others' suggest that glucose may have protective effects in focal ischemia, and since most strokes and neurosurgical procedures are associated with focal ischemic lesions, definitive recommendations must await further investigation.

Acknowledgments

The authors wish to thank Dikran Horoupian, MD, for his constructive suggestions, Gail V. Benson for her technical support, Beth Millerman for her preparation of the histologic materials, and the Puritan-Bennett Corporation for their donation of a medical gas analyzer.

References

10. Nedergaard M: Transient focal ischemia in hyperglycemic rats is associated with increased cerebral infarction. Brain Res 1987;408:79–85

Key Words • cerebral ischemia • glucose • hyperglycemia • cats
Hyperglycemia decreases acute neuronal ischemic changes after middle cerebral artery occlusion in cats.

M A Zasslow, R G Pearl, L M Shuer, G K Steinberg, R E Lieberson and C P Larson, Jr

*Stroke*. 1989;20:519-523
doi: 10.1161/01.STR.20.4.519

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/20/4/519