Insulin-Induced Normoglycemia Improves Ischemic Outcome in Hyperglycemic Rats

David S. Warner, MD; Thomas X. Gionet, MD; Michael M. Todd, MD; and Alice M. McAllister

Background and Purpose: Hyperglycemia is known to aggravate ischemic brain damage. This study sought to determine if preischemic insulin-induced normoglycemia would improve outcome in hyperglycemic rats.

Methods: Normal rats and rats with 5–7 days of streptozotocin-induced diabetes were studied. Normal rats served as either fasted normoglycemic controls or dextrose-infused (hyperglycemic) controls. In the acutely diabetic rats either no insulin was given or insulin was given at 30 or 90 minutes before ischemia so as to induce preischemic normoglycemia. All rats underwent 10 minutes of forebrain ischemia. After 5 days of recovery, motor function and histological outcome were assessed.

Results: Untreated diabetic rats and dextrose-infused control rats had greater hippocampal CA1 damage than normoglycemic control rats. In contrast, insulin-treated diabetic rats had less hippocampal CA1 damage than either untreated diabetic rats or dextrose-infused control rats. Injury in the two insulin-treated groups was not significantly different from that in the normoglycemic control group (all three groups had plasma glucose values of 120–150 mg/dl immediately prior to ischemia). Despite similar plasma glucose values (300–400 mg/dl), fewer postischemic seizures (0% versus 67%) were observed in the untreated diabetic group than in the dextrose-infused control group (p < 0.001).

Conclusions: Hyperglycemia caused by either dextrose infusion or streptozotocin-induced diabetes resulted in exacerbated ischemic brain damage. Insulin therapy to rapidly induce preischemic normoglycemia improved outcome from forebrain ischemia in the acutely diabetic rats. Glucose-infused hyperglycemic rats frequently exhibited postischemic generalized seizures while acutely diabetic rats did not. The latter results implicate some adaptive/protective mechanism associated with acute streptozotocin-induced diabetes that results in a decreased sensitivity to hyperglycemia-augmented ischemic brain damage. (Stroke 1992;23:1775–1781)

KEY WORDS • cerebral ischemia • diabetes mellitus • hyperglycemia • rats

In numerous laboratory models, hyperglycemia has been demonstrated to exacerbate ischemic brain damage.1-7 Hyperglycemia has also been associated with worsened outcome from a variety of brain insults in humans.8-12 Because hyperglycemia can be treated, such observations may have important therapeutic relevance for patients about to undergo surgery during which an ischemic insult is anticipated (e.g., carotid endarterectomy, cardiopulmonary bypass, intracranial aneurysm clipping, etc.). During such procedures, one would argue in favor of the avoidance of hyperglycemia. However, demonstrating that hyperglycemia is detrimental is not equivalent to concluding that the correction of hyperglycemia is beneficial. This may be particularly true because rapid correction of hyperglycemia with insulin administration is not risk free.

There are no human studies addressing the value of correcting preischemic hyperglycemia. However, some laboratory evidence indicates that insulin-induced hypoglycemia prior to the onset of either global or focal cerebral ischemia results in improved histological or behavioral outcome.13,14 Because reported investigations have not explored the effects of an insulin-mediated correction of hyperglycemia to a normoglycemic state, the following study was performed.

Materials and Methods

This study was approved by our institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Sasco, Omaha, Neb.) were assigned randomly to one of five groups. Rats in groups I–III received 45 mg/kg streptozotocin in saline subcutaneously 5–7 days before ischemia. No insulin was administered during this period. Before surgical preparation, each rat was fasted for 6–8 hours. The plasma glucose level was then determined via Chemstrip analysis after a tailstick (Chemstrip bG Blood Glucose Test Strips, Boehringer Mannheim Corp., Indianapolis, Ind.). Rats with plasma glucose values in the range of 250–350 mg/dl were included in the study. Approximately 85% of the rats injected with streptozotocin converted to the diabetic state, exhibiting classic signs of polydipsia, polyphagia, and polyuria. Rats in groups IV and V did not receive...
stroke but were age-matched to the rats in groups I–III. Management of plasma glucose before forebrain ischemia (FBI) for the five groups was as follows: group I (diabetic/hyperglycemia): streptozotocin 5–7 days before ischemia, 6–8-hour fast before ischemia; group II (diabetic/normoglycemia 90 minutes): as in group I, plus a subcutaneous injection of 0.3 IU/kg regular insulin (Regular Iletin I, Eli Lilly and Co., Indianapolis, Ind.) at the start of the surgical preparation (90 minutes prior to ischemia) and 0.15 IU/kg regular insulin subcutaneously 35 minutes before ischemia; group III (diabetic/normoglycemia 30 minutes): as in group I, plus an intravenous injection of 0.3 IU/kg regular insulin 30 minutes before ischemia; group IV (control/hyperglycemia): no streptozotocin, 12–16-hour fast before ischemia, intravenous infusion of 1.5–1.6 ml of 25% dextrose in 0.9% saline during the 30 minutes before ischemia; and group V (control/normoglycemia): no streptozotocin, 12–16-hour fast before onset of ischemia, no dextrose.

All rats underwent the following preparation. After being anesthetized with 4% halothane in O2, the animals were endotracheally intubated and mechanically ventilated with a delivered gas mixture of 1.0–1.6% halothane in 30% O2/balance N2. The tail artery was cannulated and mean arterial pressure (MAP) continuously recorded. Via a ventral neck incision, the common carotid arteries were isolated and encircled with suture. The right internal jugular vein was cannulated, and 35 IU heparin was given intravenously. Muscle paralysis was obtained by a 0.2-mg bolus of succinylcholine, repeated as necessary. Bipolar electroencephalographic activity was monitored from active needle electrodes inserted in the temporalis muscle bilaterally, a reference lead in the prefrontal region, and a ground lead in the tail. Finally, a 22-gauge needle thermistor was percutaneously placed adjacent to the skull and pericranial temperature was thenceforth servoregulated at 37.0±0.1°C by surface heating or cooling. The halothane concentration was then reduced to 0.5% for the remainder of the experiment. Blood samples for plasma glucose determination were obtained 45, 30, and 15 minutes before ischemia and 10 minutes after ischemia.

All rats underwent 10 minutes of FBI.15,16 Hypotension (MAP of 30±5 mm Hg) was induced with 1.75 mg i.v. trimethaphan and maintained by withdrawal and reinforcement of blood through the jugular catheter as necessary. Immediately after the onset of hypotension, the carotid arteries were occluded bilaterally with temporary aneurysm clips. Ten minutes later the vessel clamps were released, any shed blood was reinfused, and 0.27 meq NaHCO3 was given to minimize systemic acidosis. The catheters were removed and the incision sites closed with suture. Approximately 20 minutes after the ischemic insult, halothane anesthesia was discontinued and the animals were awakened. Upon recovery of spontaneous ventilation, the tracheas were extubated. The rats were then placed in a chamber containing 50% O2/50% N2 for at least 30 minutes before being returned to their cages. Over the 5-day recovery period, the animals were continuously observed for the presence/absence of generalized seizure activity and mortality/survival. No attempt was made to manipulate plasma glucose concentrations or treat convulsions during the postischemic recovery period.

Motor function tests were performed 5 days after ischemia.17 Briefly, the rats were placed on a 29×30-cm screen (grid size 0.6×0.7 cm) that could be rotated from 0° (horizontal) to 90° (vertical). The animal was placed on the horizontal screen and the screen was then rotated into the vertical plane. The time that the animal was able to hold on to the vertical screen was recorded to a maximum of 15 seconds (allowing a total of three points). Next, the rat was placed at the center of a horizontal wooden rod 2.5 cm in diameter and the time that the animal was able to remain balanced on the rod was recorded to a maximum of 30 seconds (allowing a total of three points). Finally, a prehensile traction test was administered. The time that the rat was able to cling to a horizontal rope was recorded to a maximum of 5 seconds (allowing a total of 3 points). From these three tests, a total motor score (9 possible points) was computed.

After neurological testing, the rats were anesthetized with 4% halothane in O2. Following endotracheal intubation, ventilation was mechanically controlled by a respirator delivering 3% halothane in 30% O2/balance N2. A venous blood sample was taken for the determination of plasma glucose. The brains were perfused via the ascending aorta with a 30-second flush of 0.9% saline followed by 250 ml of buffered 4% formalin (pH 7.35). The brains were allowed to stabilize at 4°C in situ overnight before removal and storage in 4% formalin.

The brains were cut coronally into 3.0-mm-thick slices and dehydrated in graded strengths of ethanol. After rinsing in xylene and embedding in paraffin, 5-μm-thick sections were serially cut and stained with celestine blue and acid fuchsin.18 Sectioning intervals were adapted to obtain specific standard levels of the hippocampus (bregma −3.3 mm and −3.8 mm) and substantia nigra pars reticulata (SNPR) (bregma −5.3 mm).19

Brain injury was initially quantified by applying a rating scale of damage to CA1 neurons of the hippocampal formation in both hemispheres at both anatomic levels. Damage was graded on a 0–3 scale (0, no observed histological changes; 1, 1–5% of neurons with pathological changes; 2, 6–50% of neurons damaged; and 3, >50% of neurons damaged)20 by one experimenter blinded to group membership. Subsequently, the numbers of viable and nonviable neurons were directly counted at ×300 in the hemisphere displaying the greater injury. Viable neurons were defined as those with normal morphology and absence of acidophilic staining. Nonviable neurons were defined as those with acidophilic staining. In the SNPR, a 0–3 damage scale identical to that used for hippocampal CA1 neurons was employed.

The data were analyzed by either one-way analysis of variance (physiological values) or the Kruskal-Wallis H test (histological and neurological values), depending on the distributions of the data. All continuous data were summarized as mean±SEM. Frequencies of postischemic seizures and death were compared between groups with the Fisher exact test. Significance was assumed at p<0.05.
Physiological values were significantly greater in all three diabetic groups compared with the frequency in any of the three diabetic groups. As intended, rats in groups II, III, and V had plasma glucose concentrations within the range of 120-150 mg/dl immediately before the onset of ischemia (Table 2). In contrast, hyperglycemia (plasma glucose concentration in the range of 300-400 mg/dl) was present in rats in groups I and IV at that time. Plasma glucose values measured on postischemic day 5 were scattered, as expected, although all diabetic groups were hyperglycemic in contrast to the control groups.

One rat died in each of the three diabetic groups. The causes of death were not identified, although in none of these rats were generalized seizures or complications from airway injuries observed. In contrast, six of 12 rats in group IV died during the postischemic interval. No rats in group V died. The frequency of death in group IV was different ($p<0.013$) from that in group V but only approached significance ($p<0.069$) when compared with the frequency in any of the three diabetic groups.

Generalized convulsions were not observed in any diabetic group nor in group V. In contrast, death in group IV was always preceded by seizures that had

### Table 1. Physiological Values for Rats Exposed to 10 Minutes of Forebrain Ischemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I ($n=12$)</th>
<th>Group II ($n=12$)</th>
<th>Group III ($n=12$)</th>
<th>Group IV ($n=12$)</th>
<th>Group V ($n=11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 min preischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>101±3</td>
<td>101±2</td>
<td>97±3</td>
<td>105±4</td>
<td>105±4</td>
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<tr>
<td>Paco2 (mm Hg)</td>
<td>39.6±0.7</td>
<td>39.7±0.4</td>
<td>40.0±0.5</td>
<td>41.1±0.6</td>
<td>41.2±0.4</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>153±5</td>
<td>159±4</td>
<td>162±3</td>
<td>163±8</td>
<td>166±7</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.42±0.01*</td>
<td>7.44±0.01*</td>
<td>7.42±0.01*</td>
<td>7.37±0.01</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>46±1</td>
<td>47±1</td>
<td>46±1</td>
<td>45±1</td>
<td>44±1</td>
</tr>
<tr>
<td>10 min postischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>81±5</td>
<td>74±5</td>
<td>70±4</td>
<td>80±6</td>
<td>77±5</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>306±7</td>
<td>286±6</td>
<td>292±7</td>
<td>301±11</td>
<td>317±4</td>
</tr>
<tr>
<td>5 days postischemia</td>
<td>278±14</td>
<td>249±14</td>
<td>263±13</td>
<td>304±20</td>
<td>312±8†</td>
</tr>
</tbody>
</table>

All values are mean±SEM. Group I, diabetic/hyperglycemia; group II, diabetic/normoglycemia 90 minutes; group III, diabetic/normoglycemia 30 minutes; group IV, control/hyperglycemia; group V, control/normoglycemia; MAP, mean arterial pressure. *$p<0.05$ different from groups IV and V when $p<0.05$ difference among groups. †$p<0.05$ different from groups II and III when $p<0.05$ difference among groups.

### Table 2. Plasma Glucose Concentrations in Rats Exposed to 10 Minutes of Forebrain Ischemia

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I ($n=12$)</th>
<th>Group II ($n=12$)</th>
<th>Group III ($n=12$)</th>
<th>Group IV ($n=12$)</th>
<th>Group V ($n=11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 min</td>
<td>376±7*</td>
<td>190±25*†</td>
<td>368±8*</td>
<td>159±9</td>
<td>158±10</td>
</tr>
<tr>
<td>30 min</td>
<td>368±16†</td>
<td>158±16</td>
<td>347±9†</td>
<td>150±7</td>
<td>143±9</td>
</tr>
<tr>
<td>2 min</td>
<td>347±12‡</td>
<td>122±8</td>
<td>129±12</td>
<td>342±25§</td>
<td>136±7</td>
</tr>
<tr>
<td>Postischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>386±9‡</td>
<td>136±11</td>
<td>141±11</td>
<td>283±10†</td>
<td>152±12</td>
</tr>
<tr>
<td>5 days</td>
<td>390±40*</td>
<td>374±30*</td>
<td>377±32*</td>
<td>181±35</td>
<td>190±14</td>
</tr>
</tbody>
</table>

All values are mean±SEM mg/dl. Group I, diabetic/hyperglycemia; group II, diabetic/normoglycemia 90 minutes; group III, diabetic/normoglycemia 30 minutes; group IV, control/hyperglycemia; group V, control/normoglycemia. *$p<0.05$ different from groups IV and V when $p<0.05$ difference among groups. †$p<0.05$ different from groups I and III when $p<0.05$ difference among groups. ‡$p<0.05$ different from groups II, IV, and V when $p<0.05$ difference among groups. §$p<0.05$ different from groups II, III, and V when $p<0.05$ difference among groups. ††$p<0.05$ different from groups II-V when $p<0.05$ difference among groups.
developed within 24 hours after ischemia. In two additional rats in this group seizures were observed, but these rats survived the full 5-day recovery interval. The 67% incidence of postischemic seizures in group IV was different from that observed in all other groups (p<0.001).

Total motor scores determined 5 days after ischemia are depicted as a cumulative fraction of each group in Figure 1. Significant differences were observed only between groups I and V (p<0.01).

In one rat in group I the entire brain was lost from histological analysis because of an error in tissue processing. Similarly, information for the SNPR from one rat in group II was lost. For the hippocampus, initial evaluation involved grading CA1 neuronal necrosis in both hemispheres. In three of the 49 brains evaluated, major interhemispheric asymmetry in histological damage scores was present. In these cases, results from the more affected hemisphere were included for data analysis. The percent dead CA1 neurons was similar at bregma -3.3 mm and -3.8 mm. Values from bregma -3.3 mm are presented in Figure 2. A main effect for treatment was present (F=5.727, p<0.0009). In group I the percent dead neurons (68±7%) was greater than in group V (47±8%, p<0.05). In contrast, the percent dead CA1 neurons was reduced in both groups II (27±8%, p<0.01) and III (32±8%, p<0.02) compared with group I. Values for groups II and III were not different from each other. Although numerically smaller, the percent dead neurons in groups II (p<0.07) and III (p<0.15) was not significantly different from that in group V. Both groups II and III and group V had less neuronal necrosis than the survivors in group IV (77±6%). The percent dead cells in group I was not different from that among the six survivors in group IV.

Results from histopathologic grading in the SNPR showed more variability within groups (Figure 3). Again, injury was less severe in group V than among survivors in group IV (p<0.02). No differences among the remaining groups were observed with the following exceptions: group I had more injury than group V (p<0.02), and injury in group II was less severe than in group IV (p<0.05).

Discussion

Evidence for hyperglycemia-induced worsening of outcome from global (and perhaps focal) ischemic brain...
damage is overwhelming. This may have unique relevance to surgical patients because cerebral ischemic insults can often be anticipated. Thus, simple maneuvers might reduce the risk of hyperglycemia-augmented ischemic brain damage in this patient population. For example, preoperative fasting has long been accepted practice. In addition, prospective studies have failed to demonstrate significant complications when intravenous dextrose was withheld during the perioperative period, and the intraoperative avoidance of dextrose-containing intravenous solutions has been widely recommended.

The question as to whether preoperative hyperglycemia should be corrected with insulin therapy is more difficult to answer. Intravenous administration of insulin carries some risk of profound hypoglycemia, which may in and of itself exacerbate ischemic brain damage. Further, it has been demonstrated that acute changes in plasma glucose concentrations, produced by either dextrose administration or insulin injection, do not accurately reflect changes in brain glucose concentrations. A normalized blood glucose concentration might lead one to erroneously assume that brain glucose concentrations have been corrected and that it is safe to proceed with a potential ischemic insult (e.g., carotid arterial cross-clamping). For these reasons, it is important to document substantial potential benefit from acute insulin therapy prior to recommending this as general practice.

An accumulating body of laboratory evidence supports the concept that insulin therapy reduces ischemic brain damage. Nedergaard and Diemer, working with rats, associated preischemic insulin-induced hypoglycemia (approximately 36 mg/dl) with a reduced infarct volume resulting from permanent middle cerebral artery occlusion. Similarly, Strong et al demonstrated improved cognitive function in normal rats rendered hypoglycemic (approximately 55 mg/dl) prior to experiencing FBI. In addition, insulin-induced hypoglycemia (approximately 65 mg/dl) has been shown to improve electrophysiological outcome from spinal cord ischemia in rabbits.

Consistent with the above investigations, we also observed improved outcome as a result of preischemic insulin therapy, although in this case plasma glucose concentrations were reduced only to normoglycemic levels. A substantial reduction in CA1 histological damage was seen and some degree of improved neurological function occurred in those diabetic rats receiving insulin. Insulin therapy was administered with two different regimens to assess if the time during which hyperglycemia was corrected influenced the efficacy of this therapy. For both histological and neurological outcome, groups II and III were essentially indistinguishable. We conclude that in this model, preischemic correction of hyperglycemia to normoglycemic values is beneficial and can be instituted as early as 30 minutes before the ischemic insult to achieve maximum benefit.

The data are also intriguing because the insulin-treated diabetic rats numerically had less hippocampal CA1 damage than did their nondiabetic normoglycemic counterparts. Although these differences did not reach statistical significance, there is precedent in the literature for concluding that insulin therapy may do more than simply correct hyperglycemia. The most salient observation is that of Voll and Auer, who showed that nondiabetic rats with clamped postischemic normoglycemia (insulin + glucose infusion) had less damage than normoglycemic noninfused controls. This indicates that insulin acts directly on the brain, independent of its glycemic effects, to reduce ischemic brain damage. Possible mechanisms of action include inhibition of neuronal firing, decreased platelet aggregation in the microcirculation, a nerve growth factor-like effect, and effects on either systemic or brain adrenergic mechanisms.

When this study was originally designed, we chose to study streptozotocin-induced diabetic rats with the belief that chronic hyperglycemia would present a more stable baseline from which plasma glucose concentrations could be manipulated with insulin administration. We presumed that the alternative—inducing acute hyperglycemia with glucose infusion followed by insulin administration—would render plasma glucose concentrations practically useless with respect to predicting brain glucose concentrations. In pilot studies with acutely diabetic rats, to our surprise, we failed to elicit the characteristic sequelae of convulsions and death in diabetic (hyperglycemic) animals. To be sure that the ischemic insult was sufficient to elicit such a response, group IV was included in the current study. Distinctly different outcomes were observed in groups I and IV despite both groups having similar plasma glucose concentrations. Postischemic seizures and frequent mortality occurred only in group IV. Postischemic seizures have been attributed to recruitment of histological injury in the SNPR as a result of preischemic hyperglycemia. However, in this experiment SNPR damage was evident in both groups I and IV, suggesting that other undefined factors differentiated the outcome between these two groups. Such factors might include differences in postischemic plasma osmolality, brain edema, or biochemical effects of streptozotocin. Because the rats in group I had the worst neurological function of surviving animals in the five groups at 96 hours postischemia, perhaps only minor influences would be necessary to account for the increased survival in that group (92%) versus that in group IV (50%).

There is some clinical evidence to support our observation that the plasma glucose concentration plays a greater role in ischemic outcome in nondiabetic subjects. Woo et al observed, in a prospective study of 304 patients, a correlation between serum glucose concentrations on admission and outcome from stroke. When the patients were stratified according to chronicity of hyperglycemia (i.e., diabetic versus nondiabetic states), only in nondiabetic patients did the correlation between serum glucose concentration and outcome persist. Pulsonelli et al have earlier reported a retrospective study of stroke in both diabetic and nondiabetic patients. While diabetic patients had worse outcomes than nondiabetic patients, no attempt was made to correlate plasma glucose concentrations with outcome in the diabetic group.

There are some limitations to the interpretation of our results. Clearly, 5–7 days of streptozotocin-induced diabetes does not mimic closely the pathophysiology of chronic diabetes mellitus. Previous work has indicated a difference in the responses of rats to chronic versus acute streptozotocin-induced diabetes and focal ischemic insult.
emia, with chronically diabetic rats faring worse. However, in that study the chronic diabetes group, being 3 months older, was not age matched to the acute diabetes group. More clearly, 5-7 days of streptozotocin-induced diabetes fails to elicit the cerebrovascular pathology expected in long-term diabetes mellitus. For example, Simpson et al. observed no differences in cerebrovascular reactivity as late as 5 weeks after induction of the diabetic state in rats. Similarly, Knudsen et al. observed altered cerebral blood flow in diabetic rats at 20 weeks but not 3 weeks after the onset of streptozotocin-induced diabetes. Extension of our findings in a rat model of chronic streptozotocin-induced diabetes would potentially be of greater relevance to the condition of clinical diabetes mellitus, in which long-standing vasculopathologic changes are commonly manifest.

In conclusion, acutely diabetic rats and non-diabetic rats were subjected to 10 minutes of FBI. Hyperglycemia induced with dextrose infusion in diabetic rats resulted in a high incidence of postischemic seizures, which was absent in diabetic rats despite their having similar plasma glucose concentrations. Acute preischemic correction of plasma glucose concentrations with insulin was effective in reducing both histological and neurological damage in diabetic rats, leading to an outcome similar to that in nondiabetic normoglycemic animals. These results indicate that preischemic insulin therapy in acutely diabetic rats improves outcome from a global ischemic insult.

References

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