Insulin Administration Protects Neurologic Function in Cerebral Ischemia in Rats

Daniel R. LeMay, BS, Lin Gehua, MD, Gerald B. Zelenock, MD, and Louis G. D’Alecy, DMD, PhD

Hyperglycemia exacerbates neurologic damage in clinical and experimental central nervous system ischemia. The purpose of our study was to determine if insulin administration before significantly alters neurologic deficit and survival after ischemia using a newly developed rat cerebral ischemia model. One hour before the onset of ischemia, 40 200–300-g Sprague-Dawley rats received intraperitoneal injections of either 1 ml normal saline or 0.4, 0.5, or 0.6 units regular insulin in 1 ml normal saline. Rats were then intubated and ventilated with 1–1.5% halothane. The aortic arch was exposed, and snares were placed on the innominate, left carotid, and left subclavian arteries. A 20-minute occlusion was begun, and anesthesia was discontinued. Baseline plasma glucose concentration was similar (p=0.48, Student’s t test) in both groups, but it subsequently was significantly lower in the 0.4 unit insulin-treated group up to 4 hours after occlusion (p=0.0035, Student’s t test). Neurologic deficit was scored on a 50-point scale (0=normal, 50=severe deficit) 1, 4, 18, and 24 hours after occlusion. In the 0.4 unit insulin-treated group the neurologic deficit score was significantly lower than in the saline-treated group 1, 4, 18, and 24 hours after occlusion (p<0.005, Student’s t test). Survival was significantly higher (p=0.001) in the 0.4 unit insulin-treated (1.7 unit/kg dose) group than in the saline-treated group. No rats died when preocclusion plasma glucose concentration was between 65 and 175 mg/dl. We conclude that insulin can be used to maintain plasma glucose concentration within a normoglycemic to mildly hypoglycemic range, which is associated with decreased behavioral neurologic deficit and increased survival following temporary cerebral ischemia. Hyperglycemia or more extreme hypoglycemia (glucose concentration less than approximately 65 mg/dl) is detrimental in this model. (Stroke 1988;19:1411-1419)

Hyperglycemia has been shown to exacerbate neurologic deficit in many experimental models of central nervous system (CNS) hypoxic-ischemic injury.7–8 Clinical studies also indicate an adverse effect on outcome when CNS ischemia occurs in the setting of hyperglycemia. Diabetic patients experiencing a stroke have significantly worse outcomes than nondiabetic stroke patients.9 Other studies have confirmed an increased mortality rate in acute stroke patients with fasting plasma glucose concentrations of >110 mg/dl.10 These findings suggest that even relatively mild hyperglycemia, whether exogenously or endogenously induced, significantly worsens postischemic neurologic outcome.

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1— Internal thoracic
recurrent
laryngeal nerve
FIGURE 1. Schematic representation of
major blood vessels supplying central ner-
vous system of rats. Snares (black bars)
are placed to minimize carotid, vertebral,
and possible subclavian collateral cere-
bral blood supply. Some blood supply to
brain still persists along the route through
aorta to intercostal arteries and retro-
grade through spinal arteries into circle of
Wills.

either an absence\textsuperscript{11} or a suppressive\textsuperscript{12} effect of
insulin on brain glucose utilization, which could be
beneficial, while other studies have demonstrated
insulin-stimulated glucose uptake\textsuperscript{13,14} and utiliza-
tion\textsuperscript{15,16} in neural tissues, which is potentially
damaging. It was unclear whether insulin pretreat-
ment would increase or decrease functional impair-
ment, and that question prompted our investiga-
tion in a newly developed rat cerebral ischemia
model.

Our objectives were 1) to develop a more consis-
tent cerebral ischemia model in rats and to adapt an
expanded evaluation of neurologic deficit, 2) to
determine the effects of insulin in this model by
monitoring plasma glucose concentration, blood
pressure, electroencephalogram (EEG), and neuro-
logic deficit, and 3) to determine if preoperative
administration of insulin significantly alters post-
ischemic neurologic deficit and survival.

Materials and Methods

Forty male Sprague-Dawley rats (2–3 months
old, 200–300 g) were individually housed in metal
cages with free access to food and water. Animal
care complied with Principles of Laboratory Ani-
mal Care and the Guide for the Care and Use of
One hour before surgery, each rat received an
intraperitoneal injection of either 1 ml normal saline
(n = 16) or 0.4 (n = 16), 0.5 (n = 6), or 0.6 (n = 2) units
regular insulin in 1 ml normal saline; 0.3 ml whole
blood was immediately taken from a tail snip for
determination of the baseline plasma glucose
concentration. Rats were weighed, then anesthetized
by placing them in a chamber containing 2% halo-
thane. Tracheal intubation with a 2.5-mm o.d. tube
8 cm long was assisted by a neonatal laryngoscope
reduced to a blade width of 7 mm. The rats were
then ventilated with an open-circuit volume respir-
ator (Phipps & Bird, Richmond, Virginia) at 90–
100 cycles/min with 1.0–1.5% halothane. Body tem-
perature was continuously monitored with a ther-
mostat inserted 3.5 cm into the rectum and was
maintained at 35 ± 0.7° C (mean ± SEM) with a heat-
ing pad. The four extremities and head were fixed to
the operating surface with tape.

A longitudinal incision was made through the skin
in the sternal region. The chest wall was incised
from the apex of the manubrium caudad along the
left sternal border, through the second rib, to the
third rib, carefully avoiding the left internal thoracic
artery. A 15-cm segment of PE 10 catheter was
placed around the innominate artery, and the free
ends were passed through a 4-cm segment of PE 160
tubing, creating a snare. The left carotid and left
subclavian arteries were isolated, and snares were
placed in a similar manner (Figure 1). Body temper-
ature was recorded, and a 0.3-ml blood sample was
taken from a tail snip for determination of preocclu-
sion glucose concentration. The three snares were
pulled and secured with a clip. Occlusion of each
vessel was verified by inspection of the snare site
and the vessel distal to the snare. Positive end-
expiratory pressure (PEEP) (8 cm water) was started
and maintained throughout the 20-minute occlu-
sion. Ventilation was maintained with room air, but
anesthesia was discontinued 1–2 minutes after occlu-
sion, and the rats remained unconscious. The snares
exited the incision cephalad to the manubrium, and
the chest was closed with 4–0 silk in three layers up
to the snares. At the end of the occlusion, the snares
were released and withdrawn, and the closure was
completed. The PEEP was discontinued, and the rat
was extubated once it maintained voluntary breath-
ing when disconnected from the ventilator.
Plasma glucose concentration was analyzed using a reflectance spectrometer (Ames seralyzer, Elkhart, Indiana) on plasma from 0.3-ml tail-snip blood samples taken at the time of treatment, before occlusion, and 1 and 4 hours after occlusion. Blood pressure was monitored from the left femoral artery using PE 50 plastic tubing filled with 40 units/ml heparinized normal saline, and EEG was recorded from needle electrodes inserted into the temporal muscles bilaterally. Blood pressure and EEG were continuously recorded on an oscillograph in three saline-treated and three 0.4
Survival was assessed and each rat was scored for neurologic deficit according to Table 1 and Figure 2 at 1, 4, 18, and 24 hours after occlusion. Overall survival was assessed 26 hours after occlusion. Surgery and neurologic scoring were performed by experimenters blinded to treatment.

Data were analyzed using Student’s unpaired two-tailed t test for plasma glucose concentration and neurologic deficit score and using Fisher’s exact test for survival (Statview 512+ software, Calabasas, California). All values are expressed as mean ± SEM.

Results

Figure 3 shows that saline- and 0.4 unit insulin-treated rats had similar baseline plasma glucose concentrations (143 mg/dl, p = 0.48), slightly higher than that in undisturbed rats, most likely due to the stress of handling and blood sampling. In addition, rats were not fasted and had been allowed free access to food and water before and after operation. Saline-treated rats demonstrated a large increase in plasma glucose concentration just before occlusion, presumably due to the additional stress of surgery and anesthesia. In 0.4 unit insulin-treated rats, plasma glucose concentration was kept from increasing and was significantly lower than in saline-treated rats up to 4 hours after occlusion.

Blood pressure and EEG were monitored in three saline-treated and three 0.4 unit insulin-treated rats during surgery and until 5 minutes after release of the occlusion. The preocclusion blood pressure was similar in these six rats, and in five of the six the response to cerebral ischemia was a consistent increase in blood pressure that was maintained throughout the occlusion (Figure 4). One saline-treated rat responded normally to the onset of cerebral ischemia and then showed a progressive decline in blood pressure. Despite being maintained on the ventilator, this rat died 90 minutes after occlusion.

The time to onset of isoelectric EEG ranged from 20 to 40 seconds, and the EEG remained isoelectric for the entire monitored period. In all 40 rats, following the onset of cerebral ischemia, there was complete pupillary dilation, which persisted throughout the occlusion. Pupil size returned to normal within 1 minute following release of the snares; however, EEG remained isoelectric to the end of the monitored period.

Overall survival 26 hours after occlusion was significantly improved in 0.4 unit insulin-treated rats compared with saline-treated rats (p = 0.001, Figure 5); one of 16 (6%) 0.4 unit insulin-treated rats died compared with 10 of 16 (63%) saline-treated...
rats. Five saline-treated rats died within 90 minutes after occlusion despite being maintained on a ventilator. Five other saline-treated rats died 20 hours after occlusion; death was usually associated with seizures. One 0.4 unit insulin-treated rat was hyperactive and four others had seizures between 17 and 26 hours after occlusion, but none of these five rats died. Seizure activity consisted of paroxysmal bouts of generalized tonic-clonic movements, with extreme hyperactive running.

Higher doses of insulin can produce excessively low plasma glucose concentrations, which can have a detrimental effect on outcome. When the rats were classified according to the units of insulin received per kilogram of body weight (insulin dose), four classes emerged (Table 2). Overall survival was maximal in the 1.7 units/kg dose class (0.4 unit insulin-treated group) and thereafter decreased as the insulin dose increased to 2.1 units/kg (0.5 unit insulin-treated group) and 2.5 units/kg (0.6 unit insulin-treated group). Two significantly lower-weight rats in the 0.5 unit insulin-treated group actually received an insulin dose in units per kilogram closer to that of the 0.6 unit insulin-treated group and therefore were included in the 2.5 unit/kg class.

Neurologic deficit score was significantly lower \((p=0.005)\) in the 0.4 unit insulin-treated group 1, 4, 18, and 24 hours after occlusion than in the saline-treated group (Figure 6). Components of the neurologic deficit score are compared in Table 3. The most significant improvements with insulin treatment occurred in consciousness and motor function.

In Figure 7, neurologic deficit scores 24 hours after occlusion were graphed against preocclusion plasma glucose concentration for all 40 rats. Neurologic deficit score in surviving rats was minimum when preocclusion plasma glucose concentration was <175 mg/dl. Six insulin-treated rats and seven saline-treated rats that died <24 hours after occlusion are indicated along the top of Figure 7. Only rats with preocclusion plasma glucose concentrations of >175 or <65 mg/dl died. Ten insulin-treated rats had plasma glucose concentrations of <35 mg/dl (which was the lower limit of sensitivity for our analyzer) either just before or 1 hour after occlusion. These 10 rats included five of the six (among 24 total) insulin-treated rats that died. As a result, we cannot determine if the cause of death among insulin-treated rats was associated with a
TABLE 2. Blood Glucose Concentration and Survival in Rat Cerebral Ischemia Model

<table>
<thead>
<tr>
<th>Insulin dose (units/kg body wt)</th>
<th>n</th>
<th>Baseline (mg/dl)</th>
<th>Before occlusion (mg/dl)</th>
<th>1 hour after occlusion (mg/dl)</th>
<th>4 hours after occlusion (mg/dl)</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml saline</td>
<td>16</td>
<td>145 ± 14</td>
<td>200 ± 6</td>
<td>131 ± 10</td>
<td>156 ± 10</td>
<td>38%</td>
</tr>
<tr>
<td>1.7 ± 0.03</td>
<td>16</td>
<td>141 ± 5</td>
<td>81 ± 8</td>
<td>60 ± 7</td>
<td>107 ± 11</td>
<td>94%</td>
</tr>
<tr>
<td>2.1 ± 0.03</td>
<td>4</td>
<td>141 ± 9</td>
<td>60 ± 6</td>
<td>45 ± 7</td>
<td>94 ± 30</td>
<td>75%</td>
</tr>
<tr>
<td>2.5 ± 0.07</td>
<td>4</td>
<td>140 ± 10</td>
<td>51 ± 7</td>
<td>39 ± 5</td>
<td>89 ± 9</td>
<td>0%</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for insulin dose and glucose concentration. Insulin was injected 60 minutes before 20-minute occlusion. Glucose concentration calculated based on a measurement lower limit of 35 mg/dl; survival 26 hours after occlusion.

Discussion

We developed a model of cerebral ischemia in rats as a modification of that model used by other investigators. We validated our model and then used it to test the effect of insulin-induced hypoglycemia/normoglycemia as a protective intervention. Global cerebral ischemia, as evidenced by isoelectric EEG, pupillary dilation, and persistent loss of consciousness, was achieved by temporary occlusion of the innominate, left subclavian, and left carotid arteries at their origin from the aorta. In contrast to the four-vessel occlusion (4-VO) model of Pulsinelli and Brierley, all branches of the vertebral arteries between the vertebral artery origin and the alar foramen are prevented from supplying blood to the CNS. In addition, collateral blood supply via the subclavian arteries is eliminated during occlusion. For these reasons we theorize that the cerebral ischemia achieved in our model is more severe than that in the 4-VO model. Occlusion is temporary but requires an open chest and mechanical ventilation. Our initial experiments, however, suggest a highly consistent neurologic deficit, which has greater morbidity and mortality than that in previously published studies.

Many laboratories using several animal models of CNS ischemia have documented that elevated plasma glucose concentration exacerbates ischemic neurologic deficit and impairs outcome. In clinical studies, Longstreth et al. have reported that following cardiac arrest, postresuscitation blood glucose concentrations were strongly and positively correlated with the degree of neurologic deficit. The admission mean blood glucose concentration of those patients who never awoke was 341 mg/dl while that in those who awoke with neurologic deficits was 262 mg/dl; among patients who awoke without any neurologic deficit mean blood glucose concentration was 251 mg/dl. Longstreth et al. have suggested that elevated blood glucose concentrations may have been secondary to increased duration of resuscitation, but in their retrospective study a cause-and-effect relation between blood glucose concentration and neurologic deficit was not excluded.

Ginsberg et al. found larger infarcts with lower plasma glucose concentrations in a photochemically induced infarction rat model. Follow-up studies by the same investigators have shown that in the middle cerebral artery occlusion model of localized infarction, hyperglycemia increases infarct size in collaterally perfused but not in end-arterial vascular territories.

Hyperglycemia is one factor that has been recognized to exacerbate injury, but the exact mechanism of glucose-associated injury is not known. Elevated tissue lactate concentration resulting from continued glucose metabolism under ischemic conditions has been implicated as the major metabolic event associated with exacerbation of ischemic neurologic injury. A previous study from our laboratory demonstrated that hyperglycemia alone is not the causative factor. In that study, we used
2-deoxyglucose to block the glycolytic flux and metabolism of glucose. Cerebral protection occurred using 2-deoxyglucose even though hyperglycemia persisted, thus indicating that it is not hyperglycemia per se or a simple osmotic effect that is damaging but more likely some metabolic event associated with hyperglycemia, possibly tissue lactate accumulation.

Many interventions have been used to reduce the severity of deficit following hypoxic-ischemic neurologic injury.²⁷ Theoretically, mild insulin-induced hypoglycemia could reduce the availability of glucose as a substrate and therefore reduce the formation of the lactate that would accumulate under the anaerobic conditions of ischemia.

Traditionally, the brain has been viewed as independent of the insulin effect of altered glucose uptake and metabolism. Of concern are studies showing that insulin stimulates glucose uptake¹³,¹⁴ and utilization¹⁵,¹⁶ in neural tissues including whole rat brain or cultured rat glial cells. Therefore, under ischemic conditions one might anticipate an adverse effect of insulin due to the local accumulation of CNS tissue lactate following increased glucose uptake. In contrast, others have shown that insulin has no effect¹¹ or a suppressing effect¹² on brain glucose utilization, which would be consistent with its protective effects in our study. One must also consider possible regional differences in the brain's response to insulin.¹²,¹⁶

Even though the effect of insulin on nervous tissue remains controversial, in our model of cerebral ischemia insulin at appropriate doses appears to protect neurologic function. Rats given 0.4 unit insulin (approximately 1.7 unit/kg body wt) showed significant improvement in neurologic deficit score compared with saline-treated rats. An important observation is that the degree of hypoglycemia is related to outcome. When the dose was increased to 2.1 and 2.5 units/kg, survival decreased (Table 2).

One hour after occlusion there was a significant improvement in the insulin-treated group compared with the saline-treated group in consciousness but not in motor function (Table 3). The lack of difference in motor function is most likely due to a decreased motivational state, which all rats exhibited even when consciousness appeared normal. This decreased motivational state may be due in part to residual effects of the anesthetic.

Figure 7 supports the conclusion that mild hypoglycemia/normoglycemia protects neurologic function and survival during and after cerebral ischemia. Increased survival may be a result of decreased neurologic deficit, but we do not exclude the possible protection of other systems. When hypoglycemia became more profound survival decreased, but rats surviving this hypoglycemia had minimal neurologic deficits, suggesting that the cause of death in hypoglycemic rats was more related to

![Figure 7. Scatter plot of neurologic deficit score 24 hours after occlusion vs. preocclusion plasma glucose concentration for 40 saline- and insulin (Ins)-treated rats. Six insulin-treated and seven saline-treated rats that died <24 hours after occlusion are shown across top. Plot supports existence of range of protection for preocclusion plasma glucose concentration.](http://stroke.ahajournals.org/)

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**Table 3. Comparison of Components of Neurologic Deficit Score in Rat Cerebral Ischemia Model**

<table>
<thead>
<tr>
<th>Component</th>
<th>Maximum score</th>
<th>Hours after occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Saline (1 ml)</td>
<td>Insulin (0.4 units)</td>
</tr>
<tr>
<td>Consciousness</td>
<td>10</td>
<td>7.6 ± 0.45</td>
</tr>
<tr>
<td>Motor function</td>
<td>17</td>
<td>14.5 ± 0.21</td>
</tr>
<tr>
<td>Cranial nerves</td>
<td>8</td>
<td>2.4 ± 0.97</td>
</tr>
<tr>
<td>Spinal nerves</td>
<td>6</td>
<td>2.9 ± 0.37</td>
</tr>
<tr>
<td>Respiration</td>
<td>9</td>
<td>2.6 ± 1.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*tp<0.05, <0.001; Student's t test.
direct hypoglycemic effects and not to ischemia-induced neurologic deficits. Hyperglycemia is associated with both increased neurologic deficits and decreased survival, which may suggest a more causal relation.\textsuperscript{6–8}

Siemkowicz and Hansen\textsuperscript{2} studied neurologic outcome following cerebral ischemia in hypoglycemic, normoglycemic, and hyperglycemic rats. They determined that hyperglycemia and hypoglycemia hamper recovery after cerebral ischemia induced by pneumonic cuff compression of neck vessels in addition to blood loss. In that study, insulin-treated rats had a preischemic mean plasma glucose concentration of approximately 34 mg/dl. In another study, Ibayashi et al\textsuperscript{28} concluded that hypoglycemia should be avoided in cerebral ischemia because hypoglycemia leads to severe metabolic disturbances and decreased survival, but this conclusion was based on a mean ± SEM ischemic plasma glucose concentration of 32 ± 21 mg/dl. These results agree with our findings that hypoglycemia of this degree is detrimental to recovery. Therefore, it may be important to maintain plasma glucose concentration above a lower critical level of approximately 65 mg/dl. Our laboratory has performed a complementary study using insulin to prevent hyperglycemia in a rat model of spinal cord ischemia with the similar result that mild insulin-induced hypoglycemia appears to protect neurologic function.\textsuperscript{29}

There are several mechanisms by which insulin could be protective in the brain. Insulin decreases hepatic glycogenolysis and gluconeogenesis and lowers plasma glucose concentration by preferentially increasing uptake into muscle and fat and therefore reduces the amount of glucose circulating and available for brain metabolism. We cannot exclude the possibility that insulin could also suppress the utilization of glucose by the brain under ischemic conditions. Alternatively, insulin may have a protective effect on the brain during ischemia by a mechanism that does not involve alterations in glucose supply or utilization. Our study does not identify any one or a combination of any of these possible mechanisms.

We have examined the effect of insulin-induced hypoglycemia/normoglycemia on neurologic outcome in a new model of cerebral ischemia in rats. We used insulin to reverse endogenous elevations in plasma glucose concentration resulting from surgery. Compared with saline-treated rats, significant protection of survival and neurologic function resulted from 0.4 unit regular insulin treatment (1.7 unit/kg dose). Survival decreased as higher insulin doses caused plasma glucose concentrations to fall below a lower critical level. We conclude that mild insulin-induced hypoglycemia/normoglycemia, above approximately 65 mg/dl, improves survival and neurologic function in this rat model of cerebral ischemia.

Acknowledgment
We thank Carol Goodenough for assistance with illustration.

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KEY WORDS • cerebral ischemia • hyperglycemia • insulin • hypoglycemia • rats
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