Insulin reduction of cerebral infarction due to transient focal ischemia


Departments of Clinical Neurosciences and Pathology, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada

Insulin has recently been shown to ameliorate damage in models of global brain ischemia. To determine whether insulin is also neuroprotective in focal ischemia, 20 rats were given 2 to 3 IU/kg insulin and 10 did not receive treatment prior to normothermic transient middle cerebral artery occlusion for 2 hours at a blood pressure of 60 mm Hg. To further elucidate whether infarction volume is influenced by variations in blood glucose levels within the physiological range, blood glucose was raised in 10 of the insulin-treated animals to levels comparable with the untreated controls. At 1-week survival, damage was assessed using quantitative neuropathological examination of 25 coronal planes. It was found that preischemic insulin lowered the mean intraischemic blood glucose level from 8.4 ± 0.2 mM (±standard error of the mean) in the control group to 3.4 ± 0.2 mM and reduced total damage (atrophy plus cortical and striatal necrosis), expressed as the percentage of the normal hemisphere, from a control of 28.5% ± 2.9% to 14.5% ± 1.6% (p < 0.005). Coadministration of glucose and insulin resulted in a mean intraischemic blood glucose level of 10.1 ± 0.5 mM, with 27.0% ± 2.4% total damage (p = 0.96, compared with control). Total ischemic damage showed an independent correlation with blood glucose levels (r = 0.67, p = 0.0018).

The findings indicate that insulin benefits focal ischemia and that reducing the blood glucose from 8 to 9 mM to the low-normal range of 3 to 4 mM with insulin dramatically reduces subsequent infarction. The data suggest that the neuroprotective mechanism of insulin action in focal middle cerebral artery occlusion is mediated predominantly via alterations in blood glucose levels. In comparison to global ischemia, focal ischemia appears to show only a minor direct central nervous system effect of insulin. In clinical situations in which transient focal ischemia to the hemisphere can be anticipated, insulin-induced hypoglycemia of a mild degree may be beneficial.

KEY WORDS • cerebral ischemia • infarction • insulin • neuroprotection
Insulin and blood glucose in focal ischemia

Focal ischemia to determine whether insulin can reduce infarction volume. Because of the potential therapeutic significance of the use of insulin and the opportunity to mimic clinical situations, blood glucose levels were manipulated only within the physiological range. Giving glucose with insulin in some animals would enable determination of the kind of mechanism involved; any benefit accruing from a mechanism involving hypoglycemia would be annulled by concurrent administration of glucose, whereas a direct beneficial effect of insulin would persist in the absence of hypoglycemia.

Materials and Methods

Animal Preparation

Thirty male Sprague-Dawley rats, each weighing between 378 and 496 g, were used in this study. Anesthesia was induced with 3% halothane in 1:1:1 mixtures of 50% O2 and 50% N2O, in rats that had been allowed free access to rat chow and water before surgery. The rats were intubated with P-205 polyethylene tubing* passed over a guide wire inserted between the vocal cords. The animals were then ventilated with 1% halothane using a Starling-type ventilator and given atropine, 0.06 mg intramuscularly, to reduce respiratory secretion.

The ventral tail artery was cannulated with P-50 polyethylene tubing and connected to a transducer.† Blood pressure was automatically recorded by computer every 5 seconds. The lateral tail vein was cannulated with P-50 polyethylene tubing and connected to an infusion of 0.9% normal saline run at 4 ml/kg/hour. The blood glucose, pH, PCO2, PO2, and hematocrit levels were monitored via a laboratory-grade glucometer. Arterial blood was sampled via an arterial line. Blood glucose was measured using glucose oxidase reagent strips and a reflectance glucometer. Core temperature was monitored via an internal thermistor regulated servo-controlled heating blanket. Ipsilateral temporalis muscle temperature was quickly brought to 37 ˚C by means of the halothane concentration to 1.75% to 2.25%. The MCA, mean arterial blood pressure was reduced to 60 mm Hg by increasing the halothane concentration to 1.75% to 2.25%. The MCA, clearly visible through the dura, was then occluded in two locations with 10-0 monofilament nylon suture, according to the method of Cole et al. The dura was left intact during the occlusion process, to avoid atmospheric exposure of the brain and artery. The location of the first MCA occlusion was just proximal to the origin of the temporalis muscle, removal of the zygoma, and reflection of the temporalis muscle and the trigeminal nerves from the temporal bone. The exposure was maintained using two self-retaining retractors; constant irrigation of warm saline was used to obviate focal hypothermia. A pneumatic craniectomy was performed using a microsurgical technique.

To reduce the variability of the infarction size in all animals, the mean arterial blood pressure was reduced to 60 mm Hg by increasing the halothane concentration to 1.75% to 2.25%. The MCA, clearly visible through the dura, was then occluded in two locations with 10-0 monofilament nylon suture, according to the method of Cole et al. The dura was left intact during the occlusion process, to avoid atmospheric exposure of the brain and artery. The location of the first MCA occlusion was just proximal to the origin of the temporalis muscle, and the second occlusion was at the level of the inferior cerebral vein.

After 2 hours of MCA ischemia, the sutures were cut and removed, the blood pressure was returned to normal, and reperfusion of the MCA was confirmed by direct visual observation. The operative site was covered with Gelfoam and the wound was closed in anatomical layers (temporalis muscle and skin) using 4-0 silk suture. The animals were monitored for a further 15 to 20 minutes to ensure that all physiological parameters had stabilized, the arterial and venous lines were then removed, and the access wound sutured with 4-0 silk. The tip of the tail was cut to allow access to venous blood in the postoperative period for blood glucose measurement. The rats were then allowed to awaken from the anesthesia.

All animals had free access to food and water in the postoperative period. Blood glucose levels were recorded 2 hours after the operation and at least four times thereafter for the first 24 hours. In addition, the weight and Bederson neurological grade were determined at 2 and 6 hours of recovery, and on the 1st and 7th postoperative days. The Bederson neurological grading scheme for focal ischemia in the rat can be briefly summarized: Grade 0 is a normal examination, Grade 1 represents a minimal deficit, and Grades 2 and 3 describe moderate and severe hemiplegia, respectively.

Insulin and Glucose Administration

The insulin used consisted of porcine/bovine crystalline zinc insulin (CZI) and longer acting zinc insulin suspension (Lente); the dosages used in these experiments were described below. The rats were randomly allocated to three groups. To distinguish any effect of insulin-induced hypoglycemia from that of insulin alone, glucose was given to one group of insulin-treated rats as follows: 10 rats in Group 1 (control) were left untreated. The 10 rats in Group 2 (insulin with moderate hypoglycemia) were treated with insulin only and were allowed to become moderately hypoglycemic (blood glucose 3 to 4 mM). The rats in Group 2 received 2.0 to 2.5 IU/kg insulin in a ratio of 1:2 CZI/Lente intraperitoneally 50 to 70 minutes prior to the onset of ischemia. We established this dose of insulin as optimum for reduction of nonfasting sugar to the defined levels during praeexperimental trials. Additional soluble insulin (CZI) was administered, if needed, during the operative procedure to maintain the blood glucose levels in the range of 3 to 4 mM. During the first 24 hours of recovery, blood glucose concentrations were maintained within this range with intraperitoneal injections of insulin as needed.

The 10 animals in Group 3 (insulin plus glucose with normoglycemia) were treated with insulin, but hypoglycemia was prevented by concurrent glucose administration. The rats in Groups 3 received 3 IU/kg insulin in a ratio of 1:2 CZI/Lente intraperitoneally 50 to 70 minutes prior to the onset of ischemia. Immediately after the insulin was administered, the animals received 2 to 4 g/kg of 50% glucose intraperitoneally, and their drinking water was supplemented with 25% glucose to reduce the fluctuations in blood glucose levels while receiving insulin. Blood glucose levels were further regulated to the 5- to 10-mM range over the postischemic period as follows. During the first 24 hours of recovery, the animals were given twice daily intraperitoneal injections of 2 IU/kg Lente insulin and supplemental intraperitoneal glucose injections to maintain blood glucose levels in the range of 5 to 10 mM. This dose of insulin was selected to be similar to that used in Group 2, and because of its demonstrated efficacy in our laboratory in global cerebral ischemia.

Analysis of Hemispheric Damage

The rats in all groups were allowed to survive for 1 week after the operation. The animals were then given 2% halothane anesthesia and killed by means of transcardiac perfusion performed for 30 seconds with saline, followed by 4% formaldehyde, phosphate-buffered to pH 7.30.

The brains were removed and cut coronally into 3-mm slices. After processing, embedding in paraffin, and serial sectioning at 6-µm thickness, slides of the brain were made at 250-µm intervals.

---

* Polyethylene tubing obtained from Clay Adams, Parsippany, New Jersey.
† Transducer, Model Gould P50, supplied by Statham, Cleveland, Ohio.
‡ Crystalline zinc insulin and zinc insulin suspension supplied by Novo Laboratories Ltd., Willowdale, Ontario, Canada.
and stained with hematoxylin and eosin. Sections encompassing the entire infarction, spaced 500-μm apart, were analyzed and referenced to the interaural line. § Each of these sections, the areas of each hemisphere and of infarcted cortex and striatum were traced by an observer (M.H.), blind to the treatment received by each rat, using a microscope and an image analysis system.

It was apparent from the differences in the areas of the ipsilateral and contralateral hemispheres (Fig. 1) that atrophy of the ischemic hemisphere had already occurred by 1 week. To measure this tissue loss, probably related mainly to necrotic tissue removal by macrophages, the total areas of the ischemic and nonischemic hemispheres were measured. Atrophy was calculated by subtracting the area of the ischemic hemisphere, including the areas of necrosis, from the nonischemic hemisphere.

Volumes of cortical necrosis, striatal necrosis, and atrophy were then determined using a three-dimensional reconstruction program,§ which integrated all x-y area measurements in the third dimension to calculate the damage volumes, taking into account the alignment of the sections along a reference point (the center of the corpus callosum). The percentage of hemispheric damage was calculated by adding tissue loss due to atrophy to that due to necrosis and dividing these by the area of the contralateral hemisphere.

Physiological parameters were assumed to be parametric variables and were compared using one-way analysis of variance (ANOVA), with Scheffé’s test for multiple comparison between groups. One-way ANOVA with Scheffé’s test was also used to compare the areas of necrosis and atrophy at all coronal planes and to compare the damage volumes. The Kruskal-Wallis test was used to compare the Bederson neurological rating scale (Fig. 2) at both 6 hours (p < 0.05) and 24 hours (p < 0.005). By 7 days of recovery (data not shown), the median score in all groups had returned to zero.

All 30 rats survived 1 week. Insulin improved their clinical restitution after ischemia. Group 2 rats treated with insulin but not glucose showed a substantial improvement in the Bederson neurological rating scale (Fig. 2) at both 6 hours (p < 0.05) and 24 hours (p < 0.005). By 7 days of recovery (data not shown), the median score in all groups had returned to zero.

One Group 1 brain was damaged during histological processing, and it was necessary to exclude one Group 2 rat from the analysis due to a large cerebral abscess. Untreated animals had a cortical infarct volume of 39.9 ± 7.3 mm³. Insulin administered to hypoglycemic rats reduced the necrotic cortical volume to 22.5 ± 3.1 mm³, and insulin given with glucose reduced the volume to 30.3 ± 5.4 mm³. Although mean infarct areas were less in both treated groups at all coronal levels examined, these volume differences (Fig. 3) were not significant.

In the striatum, insulin alone reduced the volume of striatal necrosis in the animals from 19.3 ± 3.8 mm³ to 6.8 ± 4.3 mm³.

---

**Results**

The rats receiving only insulin had lower blood glucose levels than either the controls or animals receiving insulin and glucose (Table 1), before, during, and for the 24 hours after the 2-hour ischemic episode. Intrainschematic blood glucose levels in the insulin-treated group fell within the low but physiological range (3.4 ± 0.2 mM). Animals treated with glucose plus insulin had intrainschematic glucose levels that were higher than the controls by 1.7 mM. There were no significant differences between groups in the remaining physiological parameters, except for a lower intrainschematic pH and a higher PO₂ in Group 3 (Table 2).

All 30 rats survived 1 week. Insulin improved their clinical restitution after ischemia. Group 2 rats treated with insulin but not glucose showed a substantial improvement in the Bederson neurological rating scale (Fig. 2) at both 6 hours (p < 0.05) and 24 hours (p < 0.005). By 7 days of recovery (data not shown), the median score in all groups had returned to zero.

One Group 1 brain was damaged during histological processing, and it was necessary to exclude one Group 2 rat from the analysis due to a large cerebral abscess. Untreated animals had a cortical infarct volume of 39.9 ± 7.3 mm³. Insulin administered to hypoglycemic rats reduced the necrotic cortical volume to 22.5 ± 3.1 mm³, and insulin given with glucose reduced the volume to 30.3 ± 5.4 mm³. Although mean infarct areas were less in both treated groups at all coronal levels examined, these volume differences (Fig. 3) were not significant.

In the striatum, insulin alone reduced the volume of striatal necrosis in the animals from 19.3 ± 3.8 mm³ to 6.8 ± 4.3 mm³.

---

§ Three-dimensional reconstruction program, PC3D, obtained from Jandel Scientific, San Rafael, California.

|| Stata, Version 3, obtained from Stata Corp., College Station, Texas.

---

**TABLE 1**

Blood glucose levels and insulin doses*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Insulin</td>
<td>Insulin &amp; Glucose</td>
</tr>
<tr>
<td></td>
<td>Normoglycemia</td>
<td>(Moderate Hypoglycemia)</td>
<td>(Normoglycemia)</td>
</tr>
<tr>
<td>initial insulin dose (IU·kg⁻¹)</td>
<td>0</td>
<td>2.28 ± 0.14</td>
<td>2.98 ± 0.02†</td>
</tr>
<tr>
<td>duration from insulin</td>
<td>—</td>
<td>64.0 ± 8.6</td>
<td>63.5 ± 7.3</td>
</tr>
<tr>
<td>administration to ischemia (min)</td>
<td>8.7 ± 0.25</td>
<td>6.1 ± 0.30‡</td>
<td>9.9 ± 0.44</td>
</tr>
<tr>
<td>blood glucose, mM (preischemic</td>
<td></td>
<td>3.6 ± 0.20‡</td>
<td>9.8 ± 0.84</td>
</tr>
<tr>
<td>mean)</td>
<td>8.9 ± 0.28</td>
<td>3.6 ± 0.20‡</td>
<td>9.8 ± 0.84</td>
</tr>
<tr>
<td>blood glucose, mM (intraischemic</td>
<td></td>
<td>3.4 ± 0.17‡</td>
<td>10.1 ± 0.49§</td>
</tr>
<tr>
<td>mean)</td>
<td>8.4 ± 0.18</td>
<td>3.4 ± 0.17‡</td>
<td>10.1 ± 0.49§</td>
</tr>
<tr>
<td>blood glucose, mM (postischemic</td>
<td></td>
<td>3.5 ± 0.09</td>
<td>7.0 ± 0.41**</td>
</tr>
<tr>
<td>mean)</td>
<td>5.1 ± 0.26</td>
<td>3.5 ± 0.09</td>
<td>7.0 ± 0.41**</td>
</tr>
</tbody>
</table>

* All values are given as mean ± SEM (n=1 method). Values: † = higher than Group 2 (p < 0.05); ‡ = lower than Group 1 or Group 3 (p < 0.001); § = higher than Group 1 (p < 0.01); ‖ = lower than Group 1 or Group 3 (p < 0.005); ** = significantly higher than Group 1 (p < 0.001).
4.2 mm³ (p < 0.05). No protection was seen when glucose was coadministered with insulin, with a necrotic volume of 24.2 ± 3.8 mm³.

Hemispheric atrophy (Fig. 4) was reduced by insulin alone from 54.6 ± 8.4 mm³ to 20.6 ± 5.0 mm³ (p < 0.05), but insignificantly by glucose plus insulin to 42.0 ± 8.3 mm³. A representative section illustrating the hemispheric atrophy is shown (Fig. 1).

The sum of cortical necrosis, striatal necrosis, and hemispheric atrophy was 113.6 ± 17.8 mm³ in untreated animals (Fig. 5). Insulin with moderate hypoglycemia reduced total damage to 51.9 ± 7.7 mm³ (p < 0.05). The insulin/normoglycemia group showed an insignificant reduction from control group, with a total damage volume of 96.5 ± 12.5 mm³.

When total damage was expressed as the percentage of the normal hemisphere, insulin with moderate hypoglycemia reduced damage from a control of 28.5% ± 2.9% to 14.5% ± 1.6% of the hemisphere (p < 0.005). Coadministration of glucose and insulin resulted in no significant change from untreated animals, with 27.0% ± 2.4% damage within the hemisphere between bregma −2.2 mm and bregma −14.7 mm.

To further investigate the mechanism of insulin action in focal ischemia, specifically the importance of blood glucose levels, regression analyses were done to define the relationship of the blood glucose levels to the total volume of cerebral damage. Because our previous data in transient global ischemia indicated that insulin acts at least partly via a direct effect on the central nervous system, it was necessary to control for the presence or absence of insulin in such analyses. Thus, only animals receiving insulin (Groups 2 and 3) were included. The strongest relationship (Fig. 6) was between the mean preischemic blood glucose and the total damage volume (r = 0.67, p = 0.0018), although there was also a correlation between the intraischemic blood glucose level and the total damage volume (r = 0.66, p = 0.0021).

Discussion

This study demonstrates that insulin pretreatment mitigates cerebral infarction due to transient focal ischemia, augmenting the conclusions from models of transient global cerebral ischemia. Furthermore, lowering blood glucose levels, even within the physiological range,
modulates the size of the resulting MCA infarction. Insulin with mild hypoglycemia reduced cortical infarction, with no increase in mortality. The reduction in damage volume was in fact associated with a significant improvement in the neurological restitution. This effect was seen by lowering blood glucose within the physiological range.

One study reduced blood glucose levels with insulin outside the physiological range to 2.0 mM and demonstrated a reduction in infarction size compared to animals having a blood glucose level of 28 mM. Other researchers claimed a beneficial effect from both fasting and insulin treatment; however, no consistent effect of blood glucose levels on infarction size was shown, and infarction volumes were 99 ± 26 mm$^3$ (mean ± standard error of the mean) at less than 60 mg/dl, 9 ± 6 mm$^3$ at 60 to 180 mg/dl, and 112 ± 36 mm$^3$ at more than 180 mg/dl blood glucose levels. Izumi and colleagues recently discussed the possibility of a direct neuroprotective role of insulin in focal MCA occlusion, but blood glucose levels were not a controlled variable and were occasionally, but not always, different from controls. In addition, the insulin-treated animals had a lower body temperature (up to 0.9°C) than untreated controls. After transient global ischemia, insulin has been shown to have a direct central nervous system effect in reducing cerebral necrosis. The results in this study suggest that such a direct effect, if it exists, is far less important in transient focal ischemia.

Many experimental studies examining the role of glycemia during focal cerebral ischemia have used extremely high blood glucose levels, probably seen clinically only in diabetic patients. Such high blood glucose levels undoubtedly exacerbate the detrimental ischemia-induced lactic acidosis and can also worsen cerebral blood flow. However, in our study, hyperglycemia was minimal. Blood flow was shown to be reduced by only 7% per 10 mM increase in blood glucose in nonischemic animals; thus, the mechanism of glucose modulation of ischemic damage likely involves metabolic, not blood flow effects, which supports the conclusions of others. This metabolic mechanism may involve mitigation of intraischemic acidosis by preischemic hypoglycemia, as shown in global ischemia.

The finding of a less prominent component of direct insulin action in focal as opposed to global ischemia warrants an explanation. Transient global ischemia is characterized by reperfusion after much shorter ischemic periods than focal ischemia producing infarction. Furthermore, it is accompanied by delayed neuronal death, during which time insulin might act centrally in the reperfusion period to salvage neurons. Transient focal ischemia, in contrast, involves arterial occlusion and thereby attenuates direct access of insulin to the core of the ischemic territory; transient focal ischemia is also tolerable for longer periods than global ischemia.
Conclusions

This experimental data may have major application in the management of human focal cerebral ischemia when pretreatment is possible. These findings have possible relevance to clinical situations involving transient MCA occlusion such as thromboembolism, but they could also be directly applied in neurosurgical procedures that require temporary occlusion of cerebral vessels, such as carotid endarterectomy, or clipping of cerebral aneurysms. In considering the clinical use of insulin, only moderate hypoglycemia (range 3 to 4 mM) should be employed to obviate the biochemical and neuropathological consequences of profound hypoglycemia on the brain. Blood glucose levels in the range of 3 to 4 mM or lower appear to be very well tolerated in humans with focal MCA occlusions. Reversible focal neurological deficits may occur, but these relate to reversible physiological inhibition in tissue function due to an altered energy state, accompanied by permanent changes in tissue structure. Mild hypoglycemia in the low-normal range of blood glucose levels may thus be therapeutically considered to obviate structural ischemic brain damage caused by temporary arterial occlusion.

Acknowledgments

The authors wish to thank Dr. Daniel Cole and his assistants, Susan Marcantonio and Terry Osborne, for excellent technical assistance in development of the ischemia model.

References


