The role of glucose uptake and metabolism in hyperglycemic exacerbation of neurological deficit in the paraplegic rat

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Previous studies indicate that hyperglycemia, particularly that induced by exogenous glucose administration, exacerbates neurological deficits in the rat spinal cord ischemic model. The effect of inhibition of glucose uptake (glucose transporter) and initial metabolism (hexokinase) on neurological outcome was evaluated in the present investigation using the competitive inhibitors 2-deoxyglucose (2-DG) and 3-O-methylglucose (3-OMG). Sprague-Dawley rats, weighing 200 to 300 gm each, received either 0.25, 1, or 2 gm/kg 2-DG; 2 gm/kg 3-OMG; 2 gm/kg glucose; or an equivalent volume of 0.9% saline intraperitoneally. Rats were intubated and ventilated with 1% to 1.5% halothane. The aortic arch was exposed and snares were placed on the right and left subclavian arteries and the aorta distal to the left subclavian artery. The three vessels were occluded for 10, 11, 12, or 13 minutes. Lower-extremity neurological deficits were evaluated at 1, 4, 18, and 24 hours postocclusion based on a 15-point scale (normal = 0, severe deficit = 15). Lower-extremity neurological deficits were significantly less severe in the groups treated with 2-DG (0.25 and 1 gm/kg) at 18 and 24 hours postocclusion (p < 0.05 for 0.25 gm/kg and p < 0.005 for 1 gm/kg, Student's t-test with Bonferroni correction). The lower 2-DG dose of 0.25 gm/kg did not significantly increase the plasma glucose level, suggesting that the glucose transporter was not markedly inhibited, and that the improved neurological outcome was more likely due to inhibition of hexokinase. The higher 2-DG dose of 1 gm/kg afforded protection despite significantly increasing the plasma glucose level, implying a strong inhibition of both the glucose transporter and hexokinase. Administration of 3-OMG, which only inhibits glucose uptake and not hexokinase, actually worsened the neurological deficit in a manner similar to that observed in rats treated with glucose. The authors conclude that the activity of the glucose transporter by itself does not significantly contribute to hyperglycemic exacerbation of neurological deficits. In contrast, the hexokinase step, at least in combination with the transporter and possibly alone, plays a significant role in hyperglycemic exacerbation of the lower-extremity neurological deficit in the paraplegic rat.

KEY WORDS: spinal cord ischemia • hyperglycemia • 2-deoxyglucose • 3-O-methylglucose • rat

Clinical studies have suggested a detrimental effect on neurological outcome when central nervous system (CNS) ischemia occurs in the setting of hyperglycemia. In many experimental models of CNS hypoxic/ischemic injury, including the rat spinal cord ischemia model, hyperglycemia has been shown to exacerbate neurological deficits. Additional studies have shown that insulin could be used to lower the plasma glucose level into the normal to mild hypoglycemic range with resulting improvement in neurological outcome. Therefore, glucose availability appears to play an important role in the determination of neurological outcome in the ischemic setting. It has been demonstrated, however, that it is not hyperglycemia alone or a simple osmotic effect that is detrimental but most likely a subsequent metabolic event associated with hyperglycemia, possibly tissue lactate accumulation. The exact mechanism of hyperglycemia-associated injury remains unclear, and it is not known what contribution is made by glucose uptake and metabolism to the hyperglycemic exacerbation of neurological deficit with ischemic injury. This study was designed to evaluate the effect on neurological outcome following ischemia of the competitive inhibitors 2-deoxyglucose (2-DG), which inhibits both the glucose transporter and subsequent glucose metabolism (hexokinase), and 3-O-methylglucose (3-OMG), which inhibits only the glucose trans-
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The enzyme hexokinase is much more sensitive to inhibition by 2-DG than is the glucose transporter. In theory, a low dose of 2-DG could be used to inhibit hexokinase without significantly inhibiting the transporter, whereas a higher dose would inhibit both hexokinase and the transporter. The goal of this study was to use 2-DG and 3-OMG to determine the contribution to the hyperglycemic exacerbation of neurological deficits made by carrier-mediated glucose uptake (glucose transporter) and the first step of glucose metabolism (hexokinase).

Materials and Methods

Male Sprague-Dawley rats, 2 to 3 months of age and each weighing 200 to 300 gm, were housed individually in metal cages with free access to food and water. Animal care complied with the National Institutes of Health guidelines “Principles of Laboratory Animal Care and Guide for the Care and Use of Laboratory Animals.” The rats received intraperitoneal injections of one of the following: 0.25, 1, or 2 gm/kg iso-osmotic 2-DG 30 minutes before vessel occlusion; 2 gm/kg iso-osmotic 3-OMG 120 minutes before vessel occlusion; 2 gm/kg iso-osmotic glucose 15 minutes before vessel occlusion; or an equivalent volume of 0.9% saline at the corresponding time control. As previously reported, anesthesia was induced by placing the rats in a chamber containing 2% halothane. Tracheal intubation was performed with a tube 8 cm long and 2.5 mm in outer diameter, assisted by a neonatal laryngoscope reduced to a blade width of 7 mm. The rats were then ventilated with an open-circuit volume ventilator at 100 cycles/min with 1.0% to 1.5% halothane.

Body temperature was continuously monitored with a thermistor inserted 3.5 cm into the rectum, and maintained between 35.5° and 36.5°C with a heating pad. The upper extremities and tail 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 top of the third rib, carefully avoiding (staying medial to) the left internal thoracic artery. The thymus was excised and the aortic arch was isolated distal to the snare. Blood supply to the spinal cord remains open along the route through the carotid artery to the circle of Willis and retrograde through the basilar artery into the anterior spinal artery.

The three snares were pulled and secured with a clip, thus occluding each vessel. Occlusion of each vessel was verified by inspection of the snare site and the vessel distal to the snare. A positive end-expiratory pressure (PEEP) of 12 cm H₂O was started and maintained through the occlusion period. The snares exited the incision cephalad to the manubrium, and the chest was closed in three layers up to the snares with 4-0 silk during the occlusion period. At the end of the occlusion period, the snares were released and withdrawn and the closure was completed. Anesthesia and PEEP were discontinued and extubation was performed when the rat could maintain voluntary ventilation on disconnection from the ventilator. To quantify the hindlimb neurological deficit, a neurological deficit score was assigned at 1, 4, 18, and 24 hours postocclusion (Table 1).

Plasma glucose levels were analyzed in plasma from 0.3 cc whole blood taken from a tail snip. A reflectance spectrometer was used for the 2-DG study and a glucose analyzer for the 3-OMG study. Blood samples were taken from treatment groups before treatment (control value), preocclusion, and 1 and 4 hours postocclusion.

Data analysis was performed using the Student unpaired two-tailed t-test for plasma glucose levels and the neurological deficit score with Bonferroni multiple-comparison correction (Apple Macintosh with Statview 512+ software). All average values are expressed as the mean ± standard error of the mean.

* Sprague-Dawley rats supplied by Charles River Laboratories, Inc., Wilmington, Massachusetts.
† Ventilator, Model 683, manufactured by Harvard Apparatus, South Natick, Massachusetts.
‡ Reflectance spectrometer manufactured by Ames, Miles Laboratories, Inc., Elkhart, Indiana; glucose analyzer, Model 23A, manufactured by Yellow Springs Instruments Co., Yellow Springs, Ohio.
TABLE 1
Spinal cord ischemia neurological deficit score*

<table>
<thead>
<tr>
<th>Deficit Score</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>walking with lower extremities (LE)</td>
<td>0: no evidence of deficit, 1: toes flat under body when walking but ataxia exists, 2: knuckles walk, 3: movement in LE but unable to knuckle walk, 4: no movement, drags LE</td>
</tr>
<tr>
<td>horizontal rope platform (upper extremities placed on platform edge)</td>
<td>0: grasps rope and pulls up with LE, 1: raises LE and grasps rope without pulling, 2: raises LE but cannot grasp rope, 3: does not raise LE</td>
</tr>
<tr>
<td>rotating screen†</td>
<td>0: LE grasp screen to 180° &gt; 5 seconds, 1: LE grasp screen to 180° &lt; 5 seconds, 2: LE grasp screen past vertical but not to 180°, 3: LE fall from screen past vertical (270°-180°)</td>
</tr>
<tr>
<td>wooden bar 1&quot; diameter at 45° (best of 3 trials)</td>
<td>0: LE grasp bar &gt; 10 seconds, 1: LE grasp bar 5-10 seconds, 2: LE grasp bar &lt; 5 seconds, 3: LE slide off bar without grasping</td>
</tr>
<tr>
<td>pain sensation</td>
<td>0: withdrawal to toe pinch, 1: reacts or squeals to toe pinch but does not withdraw, 2: no reaction to toe pinch</td>
</tr>
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</table>

* Numerical score assigned to a degree of neurological deficit. A score of zero indicates no deficit detected, with increasing numbers corresponding to increasing deficit.
† The rat is placed on a horizontal screen (0°) and the screen is rotated down through the vertical position (270°) to the inverted position (180°).

Results

The blood glucose levels, neurological outcome, and survival data used to select the doses for the subsequent studies are detailed first. Then the damaging effect of 3-OMG and the protective effect of 2-DG found in this investigation are described.

Dose Determination

Nonoperated Rats. Normal rats received 2-DG, 3-OMG, or saline intraperitoneally, and plasma glucose concentrations were monitored for up to 9 hours following injection to help determine the appropriate dose and timing range for pretreatment of operated rats. Nonoperated saline-treated rats demonstrated little change in blood glucose over the monitored period (Figs. 2 and 3). Figure 2 indicates that nonoperated 3-OMG-treated rats showed only mild increases in plasma glucose levels with the highest dose of 3 gm/kg. Figure 3 demonstrates that nonoperated 2-DG-treated rats showed a slight increase in plasma glucose levels with a 0.25-gm/kg dose while both the 1- and 2-gm/kg doses produced a major increase in plasma glucose content which persisted longer in the 2 gm/kg group.

Operated Rats. Table 2 suggests that the timing of treatment as well as the dose of 2-DG alters outcome and survival rate. A significant improvement in neurological outcome occurred with 2-DG at doses of 0.25 and 1 gm/kg injected 30 minutes before occlusion into rats undergoing spinal cord ischemia. In contrast, 2-DG at a dose of 2 gm/kg given at 30 or 60 minutes preocclusion or at a dose of 1 gm/kg given 60 minutes preocclusion resulted in significantly reduced survival rates. Although rats survived with a 2-DG dose of 2 gm/kg injected 15 minutes preocclusion, there was little effect on neurological outcome.

Blood Glucose Changes

Figure 4 demonstrates that saline-treated rats in both the 2-DG and 3-OMG studies showed an increase in
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<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>24-Hour Survival</th>
<th>24-Hour Neurological Deficit Score</th>
<th>Preocclusion Plasma Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>12/14 = 86%</td>
<td>14.1 ± 0.3 (12)</td>
<td>204 ± 12 (9)</td>
</tr>
<tr>
<td>2-DG: 0.25 gm/kg, 30 min preoccl</td>
<td>8/9 = 89%</td>
<td>11.1 ± 1.3 (8)</td>
<td>217 ± 5 (9)</td>
</tr>
<tr>
<td>2-DG: 1 gm/kg, 15 min preoccl</td>
<td>2/4 = 50%</td>
<td>14 ± 0 (2)</td>
<td>234 (1)</td>
</tr>
<tr>
<td>2-DG: 1 gm/kg, 30 min preoccl</td>
<td>9/11 = 82%</td>
<td>8.5 ± 1.4 (8)</td>
<td>308 ± 15 (11)</td>
</tr>
<tr>
<td>2-DG: 1 gm/kg, 60 min preoccl</td>
<td>1/5 = 20%</td>
<td>13 (1)</td>
<td>304 ± 9 (3)</td>
</tr>
<tr>
<td>2-DG: 2 gm/kg, 15 min preoccl</td>
<td>3/3 = 100%</td>
<td>14.3 ± 0.3 (3)</td>
<td>218 (1)</td>
</tr>
<tr>
<td>2-DG: 2 gm/kg, 30 min preoccl</td>
<td>0/2 = 0%</td>
<td>—</td>
<td>248 ± 13 (2)</td>
</tr>
<tr>
<td>2-DG: 2 gm/kg, 60 min preoccl</td>
<td>1/7 = 14%</td>
<td>8 (1)</td>
<td>295 ± 17 (4)</td>
</tr>
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</table>

* Twenty-four-hour survival (number surviving/total in group = % surviving), 24-hour neurological deficit score (mean ± standard error of the mean (SEM)), and preocclusion (preoccl) plasma glucose level in mg/dl (mean ± SEM). Sample sizes shown in parentheses.

plasma glucose level at the preocclusion measurement, which returned toward baseline by 1 hour postocclusion. These increases are most likely due to anesthetic and operative stress. In the 3-OMG protocol (Fig. 4 upper) the group treated with 3-OMG at 2 gm/kg showed a significant increase in plasma glucose level above that recorded in the saline-treated control group (p < 0.005), which was similar to that in rats treated with 2 gm/kg glucose. In the 2-DG protocol (Fig. 4 lower) only the group treated with 2-DG at 1 gm/kg had a significant increase in plasma glucose (p < 0.005).

Neurological Deficits

The neurological deficit score measured in this rat spinal cord ischemia model depended on the duration of the ischemic insult and how soon after the insult the evaluation of the deficit was made. The deficit increased as the duration of the ischemic insult increased. When the injury was less severe, the deficit decreased during recovery until the last evaluation made 24 hours after the insult. This is shown in Fig. 5 upper, which illustrates neurological deficit scores assigned at 1, 4, 18, and 24 hours following occlusion in saline-treated rats subjected to 10, 11, 12, and 13 minutes of vessel occlusion. In the saline-treated group there was a progressive increase in the deficit, reaching maximum levels in rats with 12 to 13 minutes of occlusion. Figure 5 lower contains the same 24-hour data as for the saline-treated group but presents the 24-hour deficit scores for 3-OMG-treated rats. With 3-OMG treatment, the deficit was close to maximum regardless of when the evaluation was made and even when the duration of occlusion was the shortest tested (rats treated with 3-OMG and not made ischemic showed no deficit). The difference in neurological deficit between saline- and 3-OMG-treated rats was most apparent for the 10-minute occlusion period at 24 hours following the insult.

Figure 6 shows rats in the 3-OMG protocol that underwent 10 minutes of occlusion and rats in the 2-DG protocol that underwent 12 minutes of occlusion. Figure 6 upper demonstrates that 3-OMG-treated rats had a worse neurological outcome (increased deficit) than the saline-treated control group in this protocol. The neurological deficit score of the 3-OMG-treated group was similar to that of the saline-treated rats. Figure 6 lower indicates that both the low and high
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FIG. 5. Dose-response curve showing total neurological deficit score after occlusion for four different durations in saline- and 3-OMG-treated rats. Error bars indicate standard error of the mean. Sample sizes are shown in parentheses. Upper: The saline-treated rats had a maximum deficit with 12- and 13-minute occlusions. Ten- and 11-minute occlusions caused less severe neurological deficits, which decreased with time up to the final evaluation made at 24 hours postocclusion. Lower: The saline-treated 24-hour data from the above panel is shown compared to 3-OMG-treated 24-hour neurological deficit scores. It was anticipated that 3-OMG would show protection; therefore, large samples were included in the 13-minute occlusion period. Additional testing was carried out at decreasing occlusion times until it was obvious that 3-OMG was detrimental to neurological outcome.

doses of 2-DG offered protection with statistically significantly better outcomes (lower deficit scores) obtained at 18 and 24 hours of evaluation compared to the saline-treated control group. As previously shown in Fig. 5, the saline-treated group subjected to 12 minutes of occlusion had a greater deficit than the saline-treated group with 10 minutes of occlusion.

Discussion

There are an increasing number of both experimental and clinical studies that support the hypothesis that hyperglycemia worsens neurological outcome during and following CNS ischemia. While the exact mechanism of hyperglycemic exacerbation of neurological deficits is not known, studies suggest that lactate accumulation may play a significant role in the ischemic injury process.

Some studies have suggested that hyperglycemia is not damaging in the setting of CNS ischemia. A study by Prado, et al., suggested that hyperglycemia did not increase the extent of cerebral infarction in rats, but this appears specific to end-arterial vascular territories. In a study by Woo, et al., no correlation was found between fructosamine or glycosylated hemoglobin levels and mortality rates for stroke patients. The authors chose fructosamine and glycosylated hemoglobin as better indicators of glucose levels at the onset of ischemia because these agents reflect medium- to long-term exposure to glucose and not short-term fluctuations as which would occur in a stress response to ischemia. Yet, it may not be simply the long- or short-term preischemic blood glucose levels that are important but instead the rise in blood glucose that occurs during and following an ischemic stress response. Hyperglycemia resulting from a stress response is rapid, and hyperglycemic blood would perfuse a low-flow ischemic region through collateral routes both during and after the acute phase of an ischemic event. If lactate is damaging, then increased lactate accumulation would depend on elevated glucose availability during and after the ischemic event when the aerobic capacity of the ischemic tissue is compromised.

Theoretically, it is the blood glucose level that exists during and following the ischemic event (with reperfusion) that is damaging, and a measure of preischemic blood glucose before a stress response would, therefore,
not necessarily correlate with extent of neurological injury. This is supported by studies that have shown that experimental neurological outcome is worsened even when hyperglycemia is induced after the acute ischemic event.\(^{15,25}\) Therefore, it would be preferable to know the time course for blood glucose changes not just before, but also during and after the ischemic event, to test the strength of the correlation between hyperglycemia and neurological outcome.

The outcome of an ischemic insult depends not only on the effectiveness of interventions but also on the duration of the ischemia. Figure 5 shows the correlation between blood glucose changes not just before, but also during and after the ischemic event, to test the strength of the correlation between hyperglycemia and neurological outcome.

Administration of 3-OMG, which reportedly inhibits only glucose uptake and not hexokinase,\(^{18,30}\) actually worsened the neurological deficit to the same degree as exogenous glucose. This may suggest that, in damaged postischemic spinal cord tissue, significant glucose uptake may occur by simple diffusion. Theoretically, tissues resistant to 10- to 13-minute periods of ischemia (including muscle and fat) would have the glucose transporter inhibited by 3-OMG with a resultant elevation of the plasma glucose level. Spinal cord tissue which is damaged by 10-minute periods of ischemia may have undergone compromise of blood-brain barrier (BBB) function which would normally limit glucose entry into the spinal cord. Thus, even though the glucose transporter is blocked by 3-OMG, simple diffusion (made possible by BBB dysfunction) may contribute to an increased flux of glucose from elevated plasma levels into the spinal cord cells. Treatment with 3-OMG may cause increased neurological deficits similar to that produced by exogenous glucose treatment (Fig. 4 upper) by first elevating plasma glucose, which then increases the glucose gradient and increases simple diffusion of glucose into compromised spinal cord tissue.

Previous studies have shown a protective effect of 2-DG in animals with cerebral ischemia.\(^{32,26}\) This study examined the effect of 2-DG with spinal cord ischemia and attempted to use a low dose to preferentially block hexokinase. It has been reported that 2-DG has an inhibition constant (K_i) for hexokinase of approximately 0.25 mM while the K_i for the glucose transporter is approximately 5 mM.\(^2\) Two different doses of 2-DG were chosen for this study such that the lower selected dose (0.25 gm/kg) would inhibit mainly hexokinase and the higher selected dose (1 gm/kg) would inhibit both the transporter and hexokinase. These 2-DG doses were chosen based on the experimentally determined effect that they had on plasma glucose level compared to saline-injected control animals. Figure 4 shows that plasma glucose levels for the group treated with 0.25 gm/kg was not significantly increased above those seen in the saline-treated animals, which is consistent with a minimal inhibition of the glucose transporter. In the 2-DG (1 gm/kg)-treated group there was a significant increase in plasma glucose level above the control level, consistent with a greater inhibition of the glucose transporter in addition to increased hexokinase inhibition. Protection of neurological function occurred in groups treated with both 2-DG doses. Possible complimentary studies would ideally use a blocker specific for only hexokinase to help confirm and separate the role of hexokinase from that of the glucose transporter. There are relatively specific hexokinase blockers like L-sorbose-1-phosphate, but synthesis is involved.\(^14\)

In contrast to treatment with 3-OMG, any glucose flux into compromised spinal cord tissue by simple diffusion bypassing the glucose transporter would be blocked from further metabolism by 2-DG inhibition of hexokinase. The increased protection observed with the larger dose of 2-DG may not be due to the additional blockage of the glucose transporter but instead to a more complete inhibition of hexokinase. Considering the suggested causal role of lactate with ischemic injury, the results of our experiments are supported in part by a study\(^15\) which examined the effects of 3-OMG and 2-DG on glucose and lactate concentrations in normal nonischemic rat brain. That study found that 2-DG (1 gm/kg) resulted in a large and prolonged decrease in brain lactate level and a significant elevation in rat brain glucose level consistent with a significant block of hexokinase. A 2 gm/kg dose of 3-OMG resulted in a decrease in normal brain glucose level and a transient mild decrease in rat brain lactate level. This was in nonischemic rat brain and one might conclude that 3-OMG might be protective in the ischemic setting. Our study found that 3-OMG worsened the neurological outcome. Although spinal cord lactate was not measured, the detrimental effect of 3-OMG with ischemia would be consistent with a significant flux of glucose by simple diffusion past a damaged BBB. This glycolytic flux would bypass the inhibited glucose transporter and continue through glycolysis with a possible elevation in lactate. The possibility that 3-OMG has a detrimental effect that is unrelated to its inhibition of the glucose transporter cannot be excluded.

Because we do not know the relative degree of inhibition that the two doses of 2-DG have on the glucose transporter and hexokinase, it is not appropriate to conclude that the lower dose of 0.25 mg/kg 2-DG inhibited only hexokinase and did not also have some effect on the glucose transporter. Therefore, we must conclude that the hexokinase step, at least in combi-
nation with the glucose transporter and possibly alone, plays a significant role in the hyperglycemic exacerbation of neurological deficits in the paraplegic rat. In contrast, the results with 3-OMG inhibition suggest that the functioning of the glucose transporter alone does not play a significant role in the hyperglycemic exacerbation of lower-extremity neurological deficits in this model. Cellular and molecular changes subsequent to hexokinase which are necessary for ischemic spinal cord damage require further investigation.

References


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