Effect of Hyperglycemia on Neuronal Changes in a Rabbit Model of Focal Cerebral Ischemia

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In clinical medicine, cerebral ischemia is frequently due to a focal, rather than global, insult. The effect of hyperglycemia in focal cerebral ischemia is not well defined. We studied the effect of hyperglycemia on neuropathologic changes in a rabbit model of focal cerebral ischemia. Rabbits were randomized to receive saline (n=12) or glucose (n=12) infusions. The left anterior cerebral and left internal carotid arteries were clipped after the infusion began. After 6 hours of occlusion, the area of severe ischemic neuronal damage in the left neocortex and striatum on two standard sections of brain was calculated and expressed as a percentage of the total area of the left cortex or striatum. The mean±SEM cortical area of severe ischemic neuronal damage was 22.1±2.8% in the glucose-treated rabbits and 34.0±4.6% in the saline-treated rabbits (p<0.05). The cortical area of severe ischemic neuronal damage was inversely correlated with plasma glucose concentration at the time of arterial clipping (p<0.05). We conclude that hyperglycemia is associated with decreased histologic neuronal injury in this model of focal cerebral ischemia and may be protective when cerebral ischemia occurs from a focal insult. (Stroke 1990;21:447-450)

Cerebral ischemic damage is an important cause of morbidity and mortality. Ischemic insults may be global (as occurs during cardiac arrest) or they may result from a focal interruption of blood flow (as occurs during stroke or some neurosurgical procedures). The pathophysiology of focal cerebral ischemia is distinctly different from that of global cerebral ischemia,1 and clinical interventions that may be beneficial in one may be ineffective in the other.

Conflicting evidence exists regarding the effects of hyperglycemia in different models of cerebral ischemia. It is generally accepted that hyperglycemia has deleterious effects during global cerebral ischemia. Morbidity and mortality2,3 as well as cerebral perfusion,4 metabolic restitution,5 and histopathologic changes,6 are adversely affected by hyperglycemia in models of global cerebral ischemia. However, in models of focal cerebral ischemia, some studies have demonstrated beneficial effects of hyperglycemia7-10 while others have demonstrated adverse effects.11-14

In a previous study of focal cerebral ischemia in cats,7 we demonstrated a protective effect of hyperglycemia against ischemic neuronal damage. Since such an effect may be species-dependent, our current study was designed to investigate the effect of hyperglycemia in a similar rabbit model of permanent cerebral artery occlusion.

Materials and Methods

After approval of the study by the local Institutional Review Board, 24 New Zealand white rabbits weighing 2.4–3.6 kg were fasted overnight with free access to water. Anesthesia was induced with halothane in oxygen by mask. Following tracheostomy, anesthesia was maintained with 0.6% halothane in oxygen (Puritan-Bennett anesthesia monitor, Westmont, Illinois). The rabbits were paralyzed with 0.1 mg kg⁻¹ pancuronium and ventilated with a Harvard ventilator (South Natick, Massachusetts). Ventilation was adjusted to maintain $P_{aCO_2}$ at 35–45 mm Hg. A femoral artery and vein were cannulated by cutdown. Arterial blood gases were measured immediately after arterial cannulation and every 1–2 hours throughout the study. Sodium bicarbonate was administered as needed to maintain a base deficit of <-5 meq/l. Saline was administered to replace estimated blood loss (3:1 by volume), and phenylephrine was administered by continuous infusion as necessary to maintain mean arterial blood pressure at 70–80 mm Hg. Rectal temperature was
TABLE 1. Physiologic Data in Rabbits Exposed to Permanent Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Time after occlusion (hr)</th>
<th>Rectal temperature (°C)</th>
<th>MABP (mm Hg)</th>
<th>Paco₂ (mm Hg)</th>
<th>PaO₂ (mm Hg)</th>
<th>Arterial pH</th>
<th>Glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
<td>37.5±0.2</td>
<td>73±2</td>
<td>37±2</td>
<td>395±29</td>
<td>7.36±0.02</td>
<td>166±6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>38.0±0.1</td>
<td>72±2</td>
<td>39±1</td>
<td>393±33</td>
<td>7.34±0.01</td>
<td>157±7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>38.2±0.2</td>
<td>74±1</td>
<td>39±2</td>
<td>416±11</td>
<td>7.37±0.02</td>
<td>157±7</td>
</tr>
<tr>
<td>Glucose</td>
<td>0</td>
<td>37.2±0.3</td>
<td>75±2</td>
<td>36±2</td>
<td>421±26</td>
<td>7.33±0.02</td>
<td>485±48*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>37.8±0.1</td>
<td>72±1</td>
<td>39±1</td>
<td>412±10</td>
<td>7.33±0.01</td>
<td>469±26*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>38.1±0.1</td>
<td>70±1</td>
<td>37±1</td>
<td>419±9</td>
<td>7.33±0.02</td>
<td>410±41*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 12 rabbits. MABP, mean arterial blood pressure.
*p<0.01 different from saline group by analysis of variance.

TABLE 2. Area of Severe Ischemic Neuronal Damage as Percent of Cortex or Striatum in Rabbits Exposed to Permanent Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Cortex</th>
<th>Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Section 1</td>
<td>Section 2</td>
</tr>
<tr>
<td>Saline</td>
<td>42.3±5.4</td>
<td>26.6±6.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>30.4±4.0</td>
<td>15.8±3.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM for 12 rabbits (except for Section 1 in glucose group, where n=11 because section from one rabbit was damaged during processing. Combined area for this rabbit was estimated by regression analysis using area of Section 2).
*p<0.01 different from saline group by Student's unpaired two-tailed t test.

The rabbits were randomized to treatment groups. The 12 control rabbits received 0.45% saline, and the 12 glucose-treated rabbits received 25% dextrose in 0.45% saline. All rabbits received an 8-ml/kg bolus over 15 minutes followed by an 8-ml/kg/hr continuous infusion of the designated fluid. Plasma glucose concentration was measured before the onset of focal cerebral ischemia and 2 and 6 hours later.

The left anterior cerebral and internal carotid arteries were exposed via a transorbital approach. The rabbits were fully monitored as described above. The left anterior cerebral and internal carotid arteries were clipped with Yasargil miniature aneurysm clips. The wound was closed with staples, and the rabbits were fully monitored as described above.

Six hours after clipping, a sternotomy was performed. The descending aorta was clamped, the aortic root was cannulated, the right atrium was opened for drainage, and 300 ml of 0.9% saline followed by 300 ml of 10% buffered formalin were infused by gravity into the aortic root. The brain was removed and fixed for a minimum of 2 weeks in formalin and then embedded in paraffin. The brains were sectioned coronally and stained with hematoxylin and eosin. Two standard sections, one at the level of the optic chiasm and the other at the level of the anterior commissure, were reviewed by an observer who was blinded to the treatment group; the results were confirmed by a second blinded observer.

Severe ischemic neuronal damage, characterized by moderate to severe neuronal shrinkage, increased nuclear basophilia, and nuclear pyknosis, was assessed in the left neocortex and striatum (caudate and putamen). In each section, the area of the cortex and striatum and the area of severe ischemic neuronal damage were calculated using a magnetic tablet and an IBM PC XT computer. Areas in the two sections were added to give a combined area.

Areas of severe ischemic neuronal damage are expressed as percentages of the total cortical or total striatal areas. Although the data did not violate conditions for parametric analysis, both parametric (Student's unpaired two-tailed t test) and nonparametric (the Mann-Whitney U test) analyses were used to compare areas of severe ischemic neuronal damage between the groups. The relation between plasma glucose concentration and area of severe ischemic neuronal damage in individual rabbits was similarly examined using both a simple linear regression model and the nonparametric Spearman's rank correlation coefficient. Significance was assumed at p<0.05.

Results

There were no significant differences in rectal temperature, mean arterial blood pressure, or arterial blood gas values (Table 1) or hematocrit (data not shown) between the groups at any time. The amount of phenylephrine infused did not differ significantly between the groups (data not shown), and there was no correlation between area of severe ischemic neuronal damage and amount of phenylephrine administered (data not shown). Plasma glucose concentration was significantly higher in the glucose-treated group at the time of clipping, and it remained significantly higher 2 and 6 hours after clipping (Table 1).

Areas of severe ischemic neuronal damage are presented in Table 2. The combined area of severe ischemic neuronal damage in the cortex of glucose-treated rabbits was significantly smaller than that in controls by Student's t test (p<0.05, Table 2); the difference was significant at p=0.06 by the Mann-Whitney U test. Plasma glucose concentration at the time of arterial
clipping was inversely correlated with combined area of severe ischemic neuronal damage in the cortex by simple linear regression ($r = 0.473$, $p < 0.05$) and by Spearman’s rank correlation coefficient ($r = -0.484$, $p < 0.05$; data not shown). There was no significant difference between the groups for area of severe ischemic neuronal damage in the striatum, for either individual sections or the combined area (Table 2).

Discussion

In this model of permanent focal cerebral ischemia, hyperglycemia was associated with a significantly decreased area of severe ischemic neuronal damage. Other studies have demonstrated that brains from hyperglycemic animals do not differ significantly from those of normoglycemic or hypoglycemic animals in concentrations of energy metabolites, in pH, or in neurohistology before the onset of ischemia. Brain glucose concentrations are increased in hyperglycemic animals as demonstrated in several studies.

Our findings are consistent with those of other studies examining the effects of hyperglycemia in animals subjected to focal cerebral ischemia. In a previous study, we found that hyperglycemia had a protective effect in a cat model; the area of pathologic injury was inversely correlated with plasma glucose concentration before middle cerebral artery (MCA) clipping. Ginsberg et al demonstrated that infarct volume was significantly decreased in hyperglycemic rats subjected to MCA clipping. In a model of unilateral carotid artery ligation combined with 15 minutes of photochemically produced ischemia, Jernigan et al reported that hyperglycemic rats in which a focal thrombotic infarct was initiated by reperfusion or significant collateral blood flow to this area resulted in increased intracellular potassium concentrations, which initiate a cascade of metabolic reactions resulting in multiple adverse effects including the uncoupling of oxidative phosphorylation in mitochondria, the activation of phospholipases resulting in membrane damage and the accumulation of free fatty acids, and the release of neurotransmitters including excitatory amino acids. In hyperglycemic animals, prevention of the initiating depolarizations and potassium shifts may protect the ischemic penumbra from irreversible damage resulting from this cascade.

In focal cerebral ischemia, a region of cortex adjacent to the severely ischemic or infarcted area exists that is characterized by a moderate (40%) decrease in tissue perfusion. This area has been referred to as the ischemic penumbra, in which neuronal damage is potentially reversible. Nedergaard and Diemer also demonstrated that hyperglycemia may be related to differences in the pathophysiologic changes in this area in rat brains 4 days after permanent MCA occlusion. The deflections were associated with transient increases in extracellular potassium concentrations. Elevated extracellular potassium concentrations result in increased intracellular potassium concentrations and subsequent depolarizations result in depolarizations. Elevated extracellular potassium concentrations are increased in hyperglycemic rats which initiate a cascade of metabolic reactions resulting in multiple adverse effects including the uncoupling of oxidative phosphorylation in mitochondria, the activation of phospholipases resulting in membrane damage and the accumulation of free fatty acids, and the release of neurotransmitters including excitatory amino acids. In hyperglycemic animals, prevention of the initiating depolarizations and potassium shifts may protect the ischemic penumbra from irreversible damage resulting from this cascade.

Nedergaard and Astrup also demonstrated that 2-deoxyglucose phosphorylation increased by approximately 200% in the infarct rim in normoglycemic but not hyperglycemic rats subjected to MCA occlusion. The deflections were associated with transient increases in extracellular potassium concentrations, which initiate a cascade of metabolic reactions resulting in multiple adverse effects including the uncoupling of oxidative phosphorylation in mitochondria, the activation of phospholipases resulting in membrane damage and the accumulation of free fatty acids, and the release of neurotransmitters including excitatory amino acids. In hyperglycemic animals, prevention of the initiating depolarizations and potassium shifts may protect the ischemic penumbra from irreversible damage resulting from this cascade.

The actual mechanisms by which glucose blocks depolarizations and hypermetabolism in the ischemic penumbra are unknown. Perhaps hyperglycemia alters membrane physiology or prevents glycopenia where perfusion is decreased and glucose utilization is increased. The protective effects of hyperglycemia do not appear to be mediated by changes in osmolality. Although serum osmolality was not measured in our study, previous work by Ginsberg et al found that rats pretreated with mannitol had a constant elevated serum osmolality that did not occur
in saline- or dextrose-treated rats. Furthermore, mean infarct volume was actually larger in the group pretreated with mannitol than in the saline-pretreated controls, a result opposite that found in hyperglycemic rats. These data suggest that the reduction of infarct volume in hyperglycemic animals is not osmotically mediated.

Assessment of early ischemic damage has been used by a number of other investigators in various animal models of focal cerebral ischemia. The advantage of evaluating early ischemic damage instead of established cerebral infarction is that critical cardiovascular and systemic variables can be continuously monitored throughout the survival period. Variations in these parameters can produce significant differences in the size of ischemic lesions. It remains to be established if the decreased area of severe ischemic neuronal damage in the cortex of our hyperglycemic rabbits at 6 hours will be reflected in a decreased volume of cerebral infarct at 24 or 48 hours.

In summary, in our model of permanent focal cerebral ischemia, hyperglycemia was associated with a decreased area of severe ischemic neuronal damage. Differences in the pathophysiologic changes at the infarct rim in the cortex may underlie the protective effects of hyperglycemia in this model. Further studies are needed to examine the long-term effects of hyperglycemia on neuropathology, brain physiology, and neurologic function.

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References


Key Words • cerebral ischemia • hyperglycemia • rabbits
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