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)
maintained at 37–38°C with a warming blanket and a heat lamp.

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. Approximately 1 hour after the fluid bolus, the 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, 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

### 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>Paco(_2) (mm Hg)</th>
<th>PaO(_2) (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±1</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.
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 \((r=-0.484, p<0.05; \text{data not shown})\). 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 in which a focal thrombotic infarct was photochemically produced. In a model of unilateral carotid artery ligation combined with 15 minutes of hypoxia, Jernigan et al reported that hyperglycemic rats had lower morbidity and mortality than normoglycemic controls.

In contrast, several studies have demonstrated that hyperglycemia has no effects or detrimental effects in models of focal cerebral ischemia. Many of these studies differ in model design, and the results suggest that dynamics of the cerebral circulation are important in determining the impact of hyperglycemia. Nedergaard and Diemer reported that acute hyperglycemia had no effect on infarct volume in a rat model of permanent MCA occlusion; however, when transient MCA occlusion was followed by reperfusion, hyperglycemia was associated with increased infarct volume. Prado et al noted similar results in two models of focal cerebral ischemia. In cortex receiving collateral circulation, there was a strong linear correlation between preischemic plasma glucose concentration and infarct volume; however, in regions not receiving collateral blood flow, hyperglycemia did not increase infarct volume compared with normoglycemic controls. Venables et al and Marsh et al noted adverse effects of hyperglycemia when focal cerebral ischemia was followed by reperfusion. Thus, topography of the cerebral circulation, or restitution of blood flow, appears to influence the impact that hyperglycemia has in models of focal cerebral ischemia.

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 examined the histopathologic changes in this area in rat brains 4 days after permanent MCA occlusion. In normoglycemic controls, there was a wide zone of neuronal damage adjacent to the infarct, but in hyperglycemic rats minimal damage was found in this area. In the ischemic penumbra, there was an inverse relation between neuronal damage and plasma glucose concentration. It is possible that the smaller area of severe ischemic neuronal damage in our study is due to preservation of neurons in this region of the cortex.

Significant differences in the pathophysiology of the infarct periphery further suggest that hyperglycemia is protective in this area. Nedergaard and Astrup demonstrated recurrent transient deflections in DC potential 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. Elevated extracellular potassium concentrations and subsequent depolarizations result in increased intracellular calcium 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.

Nedergaard and Astrup also demonstrated that 2-deoxyglucose phosphorylation increased by approximately 200% in the infarct rim in normoglycemic but not hyperglycemic rats. Preventing this hypermetabolism may also provide protection in the ischemic penumbra. Thus, the beneficial effects of hyperglycemia may be related to differences in the pathophysiologic changes in the infarct rim. Reperfusion or significant collateral blood flow to this area may modify these protective effects and explain why hyperglycemia has different consequences in different models.

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 cardiovasculary 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

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