Brain Perfusion in Acute and Chronic Hyperglycemia in Rats

George E. Kikano, MD, Joseph C. LaManna, PhD, and Sami I. Harik, MD

Recent studies show that acute and chronic hyperglycemia cause a diffuse decrease in regional cerebral blood flow and that chronic hyperglycemia decreases the brain L-glucose space. Since these changes can be caused by a decreased density of perfused brain capillaries, we used 30 adult male Wistar rats to study the effect of acute and chronic hyperglycemia on 1) the brain intravascular space using radioiodinated albumin, 2) the anatomic density of brain capillaries using alkaline phosphatase histochemistry, and 3) the fraction of brain capillaries that are perfused using the fluorescein isothiocyanate-dextran method. Our results indicate that acute and chronic hyperglycemia do not affect the brain intravascular space nor the anatomic density of brain capillaries. Also, there were no differences in capillary recruitment among normoglycemic, acutely hyperglycemic, and chronically hyperglycemic rats. These results suggest that the shrinkage of the brain L-glucose space in chronic hyperglycemia is more likely due to changes in the blood–brain barrier permeability to L-glucose. (Stroke 1989;20:1027-1031)
cures to minimize variations in plasma glucose levels within each group.

For the measurement of regional brain albumin space, five control, five acutely hyperglycemic, and five chronically hyperglycemic rats were anesthetized with 400 mg/kg i.p. chloral hydrate, and cannulas were inserted into a femoral artery and vein. The skin was infiltrated with local anesthetic solution and sutured. The rats were restrained in padded plastic casts after they recovered from anesthesia and were allowed to stabilize. Experiments were performed 3–4 hours after surgery when the rats were fully awake. Body temperature was monitored and maintained at 37°C by a rectal thermister probe connected to an infrared heating lamp. The arterial cannula was used to sample blood and plasma for hematocrit, glucose, pH, and gas determinations. Thirty microcuries of iodine-125–labeled human serum albumin was injected intravenously into each rat. Thirty minutes later, arterial blood samples were taken for the measurement of glucose levels and the radioactive content of the plasma, and the rat was decapitated. The brain was rapidly removed, and bilateral samples of the frontal and parietal cerebral cortex, hippocampus, cerebellum, and striatum were weighed and their iodine-125 content was measured. The albumin (and therefore, vascular) space (in microliters per gram) was determined by subtracting the iodine-125 content of the brain samples (in counts per minute per gram) by the iodine-125 content of arterial plasma (in counts per minute per microliter).

In a separate experiment using two control rats, one acutely hyperglycemic rat, and one chronically hyperglycemic rat, we determined if radioiodinated albumin was restricted to the vascular space or if it spilled across the blood–brain barrier into the brain. This was accomplished by injecting radioiodinated albumin intravenously, and after 30 minutes the rats were perfused with heparinized saline via the external jugular vein. The right atrium of the heart was free of blood, perfusion was quickly removed, and the brain was quickly removed, frozen in Freon at −40°C, and stored at −80°C. Two-micron-thick coronal sections of the frozen brain were cut by a cryostat microtome at −25°C. Sections were transferred to gelatinized slides and stored in the dark at 4°C. Three brain regions were studied, the dorsal and lateral frontal cerebral cortex (approximately 12 mm anterior to the interaural line), the dorsal and lateral anterior parietal cortex (approximately 6 mm anterior to the interaural line), and the striatum (approximately 10 mm anterior to the interaural line).

Fluorescent photomicrographs onto black and white film were obtained from air-dried sections with a ×16 objective. Then, an interference–contrast photograph of the same region was taken. Eighteen nonoverlapping fields of each region in each brain, based on at least three sections cut 150 μm apart, were recorded. The tissue sections were then stained for alkaline phosphatase by the following procedure. The sections were incubated for 30 minutes at 37°C in a freshly made fast blue RR solution (1.3 g/l) in a sodium metaborate buffer, pH 11, in the presence of magnesium sulfate and α-methylphosphate. Sections were postfixed in 1% acetic acid for 1 minute, washed, dried, and covered. Corresponding regions were located by interference–contrast microscopy so that light photographs could be taken of each field now stained for alkaline phosphatase that had been previously photographed for fluorescence. Contact prints were then made from all the negatives. An image analysis system was used to determine the simple characteristics of the number of alkaline phosphatase profiles per unit area that were <20 μm in diameter and with no more than a 2:1 aspect for largest versus smallest diameter. The corresponding fluorescent frame was then superimposed, and the number of alkaline phosphatase profiles that were associated with fluorescent spots were counted and compared with those not associated with fluorescence. The image analysis was done by a technician trained to recognize the important features in the photographs but who was uninformed of the experimental conditions; the technician handled the data in coded form only. With this method, the density of brain capillaries in each region was calculated (number of capillaries per square millimeter) and the fraction of perfused capillaries was determined.

Results

The physiological data for the three groups are presented in Tables 1 and 2. Mean plasma glucose concentration in control rats was approximately 10
TABLE 1. Physiological Variables in Control and Hyperglycemic Rats Used for Determination of Brain Albumin Space

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=5)</th>
<th>Acute (n=5)</th>
<th>Chronic (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mM)</td>
<td>10±2</td>
<td>28±2</td>
<td>27±2</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>333±16</td>
<td>345±18</td>
<td>232±14*</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47±1</td>
<td>49±1</td>
<td>47±1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.37±0.01</td>
<td>7.27±0.02</td>
<td>7.34±0.02</td>
</tr>
<tr>
<td>PAO2 (mm Hg)</td>
<td>92±3</td>
<td>88±2</td>
<td>88±2</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>35±2</td>
<td>38±4</td>
<td>37±1</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.01 different from other groups by Student's t test, two-tailed and with Bonferroni modification.

mM, while that in the acute and chronic hyperglycemia groups was approximately three times higher. We previously determined that streptozotocin-induced chronic hyperglycemia for 2 weeks had no effect on the water content of the cerebral cortex.10 Rats with chronic hyperglycemia were distinguished by their polyuria and weight loss, but arterial blood gases and pH were comparable in all groups.

The albumin space was not significantly altered in acute and chronic hyperglycemia except in the frontal cortex, where it was increased in chronic hyperglycemia (Table 3). When rats were perfused to wash out intravascular radioiodinated albumin, the iodine-125 content of the brain was negligible in all groups, indicating that acute and chronic hyperglycemia do not cause substantial extravasation of albumin across the blood–brain barrier, at least during the 30 minutes of our experiment.

The total capillary density in the various regions of the brain was not significantly altered in acute or chronic hyperglycemia (Figure 1). Also, the fraction of perfused capillaries (which ranged from approximately 70% in the frontal cortex to approximately 80% in the parietal cortex, with an intermediate ratio in the striatum) was not significantly different from control in acute or chronic hyperglycemia (Figure 1). This rules out both a decrease in the total anatomic density of brain capillaries and the possibility of decreased recruitment of brain capillaries in hyperglycemia.

Discussion

Our experiments were initially designed to explain the changes in the L-glucose space in hyperglycemia.6 Increased L-glucose space in acute hyperglycemia can easily be attributed to increased noncarrier-mediated and nonstereospecific diffusion of glucose across the blood–brain barrier when blood glucose levels are high. However, the marked shrinkage of the L-glucose space in chronic hyperglycemia is more difficult to explain. It could be caused by either diminution in the density of perfused capil-

TABLE 2. Physiological Variables in Control and Hyperglycemic Rats Injected With Fluorescein Isothiocyanate-Dextran for Determination of Brain Capillary Density

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=6)</th>
<th>Acute (n=5)</th>
<th>Chronic (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mM)</td>
<td>9±2</td>
<td>26±2</td>
<td>31±2</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>347±9</td>
<td>373±7</td>
<td>288±13*</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>48±1</td>
<td>49±2</td>
<td>45±1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.34±0.02</td>
<td>7.36±0.01</td>
<td>7.36±0.01</td>
</tr>
<tr>
<td>PAO2 (mm Hg)</td>
<td>99±3</td>
<td>88±3</td>
<td>90±2</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>38±2</td>
<td>38±1</td>
<td>39±1</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.01 different from other groups by Student's t test, two-tailed and with Bonferroni modification.
laries in the brain or by changes in the membrane characteristics of brain endothelial cells, with the net result being restriction of glucose diffusion. The first explanation appeared attractive in view of the decreased cerebral blood flow in hyperglycemia, and in view of the reported decreased density of brain capillaries in rats after 1 year of streptozotocin-induced diabetes. The lack of a major difference in cerebral blood flow between rats with chronic and acute hyperglycemia argues against this hypothesis, especially since the 1-glucose space is increased in acute hyperglycemia and decreased in chronic hyperglycemia.

We reasoned that if the effect of chronic hyperglycemia on the brain 1-glucose space was secondary to its effect on the intravascular component of that space, then results similar to those obtained with 1-glucose should be obtained with tracers that are known to remain within the brain vascular space. However, if the 1-glucose space shrinkage in chronic hyperglycemia is caused by changes in the permeability of biologic membranes to glucose, then such changes would not be detected by markers of the vascular compartment.

The results of our radioiodinated albumin experiment indicate that, if anything, rats subjected to streptozotocin-induced chronic hyperglycemia had a higher, rather than a lower, albumin space, at least in the frontal cortex (Table 3). This increase in the albumin space is probably not due to leakage of albumin across the blood–brain barrier since in another group of four rats that were treated in the same manner but subjected to transcardiac brain perfusion before sampling of the brain, we found that the amount of tracer remaining in the brain was negligible. This assures us of the veracity of our contention that the vascular space is the most important determinant of the regional brain albumin space in all groups of experimental rats. Our findings are seemingly at variance with those of Stauber et al, who showed extravasation of endogenous plasma albumin, but not of IgG or complement C3, into the cerebral cortex in streptozotocin-induced diabetic rats. However, our results are compatible with the report of Lorenzi et al that no changes in the permeability of the blood–brain barrier to sucrose, inulin, or horseradish peroxidase occur in streptozotocin-induced diabetic rats. Also, Knudsen et al reported no change in the brain sucrose space but found decreased permeability of the blood–brain barrier to sodium ions in diabetic rats. Methodologic differences probably account for the discrepancy between the results obtained by Stauber et al on one hand and those of Lorenzi et al, Knudsen et al, and ourselves on the other hand. Stauber et al used immunohistochemical methods to demonstrate vascular leakage of endogenous plasma albumin into the brain over 2 weeks, whereas in the other studies markers of the vascular space were allowed to circulate for much shorter periods. The finding by Knudsen et al of a discrepancy between the blood–brain barrier’s permeability to sucrose and its permeability to sodium ions supports our suspicion that specific alterations in brain endothelial membranes occur in diabetic rats.

The density of brain capillaries, that is, the number of alkaline phosphatase–positive profiles per square millimeter, in the frontal and parietal cortex and striatum was not altered by chronic hyperglycemia compared with either control normoglycemia or acute hyperglycemia. Likewise, the fraction of total capillaries that were filled with FITC-dextran was also not significantly altered by acute or streptozotocin-induced chronic hyperglycemia (Figure 1). Our finding of filling of 70–80% of the brain capillaries is in closer agreement to the 50–60% reported by Weiss using the same method than to the complete filling reported by Klein et al. Our method has the significant advantage that each alkaline phosphatase–positive profile can be individually examined to determine if it contains fluorescent material, but our method might underestimate the number of capillaries due to incomplete alkaline phosphatase staining. We estimate this latter error to be <10% because in separate experiments we showed that the injection of India ink into either the arterial or venous sides of the brain circulation filled vessels that were associated with alkaline phosphatase–positive profiles in all but approximately 5% (G.E. Kikano, J.C. LaManna, and S.I. Harik, unpublished observations). Thus, we believe that under our conditions of alkaline phosphatase staining, the criticism of Klein et al concerning incomplete alkaline phosphatase staining does not apply.

Our findings of a lack of shrinkage of the albumin spaces or of a decrease in capillary density during acute or chronic hyperglycemia suggests the following. First, the brain intravascular volume is not affected by hyperglycemia, at least not in short-term experiments. Second, the marked decrease in the brain 1-glucose space in rats with chronic hyperglycemia must be due to a decrease in the noncarrier-mediated diffusion of glucose across biologic membranes in this condition. Third, given the decreased regional cerebral blood flow and the unchanged vascular brain volume in hyperglycemia, one must assume increased transit time in brain vasculature in hyperglycemia. Whether this is the result of altered rheology of the blood or due to an effect of hyperglycemia on vascular endothelium remains to be determined. Fourth, our results do not support the hypothesis that chronic hyperglycemia is associated with a decrease in the anatomic density of brain capillaries, nor is it associated with decreased recruitment of perfused brain capillaries.

It is generally accepted that the microvasculature bears the brunt of the pathology in diabetes mellitus and that it is responsible for many of the pathophysiologic phenomena encountered in this malady. The evidence suggesting that brain microvessels are less permeable to 1-glucose may be indicative of struc-
tural or biochemical alterations in brain microvessels that occur in chronic hyperglycemia. Whether these changes are related to the vascular pathology that is seen in diabetes in a cause-and-effect relation or whether this is a protective mechanism designed to limit the entry of glucose from the blood to the brain must await further investigations.

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References

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