Effect of Hemodilution on Regional Cerebral Blood Flow During Chronic Hyperglycemia in Rats

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Regional cerebral blood flow decreases during chronic hyperglycemia, a condition frequently associated with elevated hematocrit. To test the hypothesis that an elevated hematocrit is responsible for the reduced regional cerebral blood flow during chronic hyperglycemia, I used isovolemic hemodilution to normalize the hematocrit in seven normoglycemic and seven streptozotocin-treated (hyperglycemic) rats. Regional cerebral blood flow was measured in 28 awake, restrained rats (14 normoglycemic and 14 hyperglycemic) using [14C]iodoantipyrine and dissection of 17 brain regions. Hemodilution lowered the hematocrit by 6 units (13%) and increased the average cerebral blood flow by 14%. Chronic hyperglycemia did not elevate the hematocrit, but it decreased the average cerebral blood flow by 12% and that in nine nontelencephalic brain regions by 17%. This effect was independent of changes in hematocrit caused by hemodilution. The reduced regional cerebral blood flow during chronic hyperglycemia is not caused by elevated hematocrit. (Stroke 1990;21:1072-1076)

Regional cerebral blood flow (rCBF) decreases during acute^1,2 and chronic^3,5 hyperglycemia. The cause of this reduction is not known, but a number of possible mechanisms have been tested. Glucose could decrease cerebral blood flow (CBF) by increasing plasma osmolality. Acute infusion of hyperosmotic solutions can affect determinants of CBF such as perfusion pressure, vascular smooth muscle tone, and blood viscosity. However, measurements of rCBF during acute infusions of glucose and mannitol indicate that the osmotic load produced by acute hyperglycemia cannot explain the observed decrease in rCBF. Alternatively, because of the close coupling of rCBF to the regional rate of energy metabolism, hyperglycemia could indirectly decrease CBF by decreasing the metabolic rate of the brain. Again, direct measurements of glucose utilization during acute and chronic hyperglycemia do not support this mechanism. More recently, measurements of the anatomic density of brain capillaries and the fraction of perfused capillaries indicate that they do not change during chronic and acute hyperglycemia, excluding these factors as possible causes for the reduction of rCBF during hyperglycemia.

Close inspection of reports of reduced rCBF during acute and chronic hyperglycemia suggest that elevated hematocrit may explain this effect. Acute hyperglycemia induced by the intraperitoneal injection of glucose and chronic hyperglycemia induced by treatment with streptozotocin are frequently associated with increased hematocrit. Hematocrit is a major determinant of whole blood viscosity and, along with hemoglobin concentration and oxyhemoglobin affinity, determines the oxygen carrying capacity of the blood. These factors contribute to an inverse relation between hematocrit and CBF. Using hemodilution to normalize the hematocrit in normoglycemic and streptozotocin-treated rats, I tested the hypothesis that an elevated hematocrit is responsible for the reduced rCBF during chronic hyperglycemia.

Materials and Methods

I used 28 male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, Mass.) weighing 250–300 g. The rats had free access to food and water before surgery. Chronic hyperglycemia was induced in 14 rats by the injection of 60 mg/kg i.v. streptozotocin 20–38 days before rCBF was measured. The 14 concurrently prepared normoglycemic control rats were housed for similar intervals.

The rCBF was measured in awake, restrained rats using procedures in accordance with the guidelines of the Milton S. Hershey Medical Center for the care of...
experimental animals. Surgery was performed using halothane/N2O anesthesia. Anesthesia was induced with 3% halothane in 70% N2O/30% O2 and maintained with 0.6% halothane. Skin incisions were infiltrated with 1% procaine and swabbed with 5% xylocaine ointment. Polyethylene catheters were placed in both femoral arteries and one femoral vein. A rectal thermistor probe was placed, and a heating lamp was used to maintain body temperature. Each rat was restrained by a plaster cast placed around the hip girdle and hind legs. The head was placed in a guillotine, and the apparatus was draped to prevent visual distraction of the rat. One hour was allowed for recovery from anesthesia, at which time the rats were awake, alert, and calm. Pulse rate and arterial blood pressure were continuously measured from one arterial catheter. Blood samples were obtained from the second arterial catheter to measure respiratory gas tension, pH, hematocrit, plasma osmolality, and plasma electrolyte concentration just before rCBF was measured. Blood from this catheter was also used to determine the appearance function of radiolabeled tracer after injection.

After 1 hour of recovery, the hematocrit was measured and a volume (V) of arterial blood was withdrawn into a heparinized syringe according to the formula: $V = (BV \times WT) [1 - (HCT_i/HCT_f)]$, where BV is the rat blood volume fraction in milliliters per gram, WT is the body weight in grams, and HCT is the initial (i) and desired (d) hematocrit. The value 0.05 was used for BV and the value 40% was used for HCT. The whole blood was centrifuged. To lower the hematocrit, the plasma was diluted with normal saline containing glucose and plasma was discarded and the erythrocytes were mixed with normal saline containing glucose and then reinjected (sham hemodilution). The average volume of blood withdrawn was 2.9 ml for normoglycemic rats and 2.2 ml for hyperglycemic rats. The interval between blood withdrawal and reinfusion was 10 minutes. Mean arterial blood pressure decreased during this interval, but not to < 100 mm Hg, and returned to baseline upon reinfusion. rCBF was measured 30 minutes later.

The rCBF was measured using the diffusible indicator [14C]iodoantipyrine (60 mCi/mmol) and methods previously described. Briefly, 40 μCi of tracer in normal saline was injected intravenously at 17 μl/sec. Freely flowing drops of arterial blood were pooled at 2-second intervals, and aliquots were prepared for scintillation counting. After 20 seconds of tracer exposure, the rat was decapitated and the brain was quickly dissected. Tissue samples from 17 brain regions (nine nontelencephalic and eight telencephalic) were transported in a humidified chamber, rapidly weighed, and solubilized for scintillation counting. rCBF was calculated using equations presented by Sakurada et al.

Pao2, Paco2, and arterial pH were measured at 37° C (BMS 3 Mk 2, Radiometer America, Inc., Westlake, Ohio). Plasma glucose concentration was measured by an automated glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Inc., Fullerton, Calif.). Plasma osmolality was measured by dew-point temperature depression (model 550, Wescor Inc., Logan, Utah). Plasma Na+ and K+ concentrations were measured by flame photometry (model 143, Instrumentation Laboratory, Lexington, Mass.). Hematocrit was measured using blood in 20-μl capillary tubes centrifuged at 13,500g for 3 minutes.

Rats were allocated in an alternating fashion to the streptozotocin-treated or the normoglycemic control group from lots of littermates received from the supplier. Rats from these two groups alternately underwent hemodilution or sham hemodilution. The intent of the experimental design was to compare rCBF between subgroups of normoglycemic and hyperglycemic rats with equal hematocrits. Because regional variations in rCBF have been measured during hyperglycemia, rCBF was compared in the 17 brain regions combined and in the nine nontelencephalic brain regions combined and the eight telencephalic brain regions combined. Physiological parameters were compared using analysis of variance (ANOVA) and t tests. rCBF was compared using a repeated-measures ANOVA (GLM procedure, SAS Institute, Inc., Cary, N.C.) using the rat as the unit of analysis. Rats were grouped by treatment in a 2×2 factorial design contrasting normal and high plasma glucose concentrations or normal and low hematocrits. The replication factor was individual brain regions, rather than time, to account for the possible interdependency of rCBF in different regions of the same brain.

Results

Treatment with streptozotocin produced a three-fold elevation in plasma glucose concentration, a failure to gain weight, and a lower resting mean arterial blood pressure. Mean±SD body temperature was 36.7±0.2° C in all subgroups. Before hemodilution mean±SD hematocrit was 45±2% in the normoglycemic control group and 46±3% in the streptozotocin-treated group. Normoglycemic and hyperglycemic rats with sham hemodilution had equal mean hematocrits of 47% before rCBF measurement. Hemodilution reduced hematocrit by 6 units, producing a mean hematocrit of 41% in both normoglycemic and hyperglycemic rats. The physiological parameters of all subgroups are shown in Table 1.

The intent of the experimental procedure was to normalize an expected difference in hematocrit between streptozotocin-treated and normoglycemic control rats. However, streptozotocin treatment did not produce the expected increase in baseline
TABLE 1. Physiological Parameters in Rats Before Measurement of Regional Cerebral Blood Flow

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n</th>
<th>Weight (g)</th>
<th>MABP (mm Hg)</th>
<th>Pulse (beats/min)</th>
<th>Arterial pH</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>[Na⁺] (meq/l)</th>
<th>[K⁺] (meq/l)</th>
<th>Hct (%)</th>
<th>Plasma glucose concentration (mmol/l)</th>
<th>Plasma osmolality (mmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control hematocrit</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>10</td>
<td>7.395±23</td>
<td>132±5</td>
<td>405±45</td>
<td>7.44±0.03</td>
<td>91±4</td>
<td>37±3</td>
<td>136±4</td>
<td>3.9±0.2</td>
<td>47±3</td>
<td>11±2</td>
<td>289±7</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>10</td>
<td>7.278±26</td>
<td>116±10*</td>
<td>383±26</td>
<td>7.35±0.04*</td>
<td>92±7</td>
<td>36±1</td>
<td>136±4</td>
<td>4.0±0.4</td>
<td>47±1</td>
<td>33±5*</td>
<td>317±9*</td>
</tr>
<tr>
<td>Hemodilution</td>
<td></td>
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</tr>
<tr>
<td>Normoglycemic</td>
<td>10</td>
<td>7.409±24</td>
<td>128±5</td>
<td>417±50</td>
<td>7.42±0.02</td>
<td>94±6</td>
<td>39±2</td>
<td>136±2</td>
<td>4.2±0.2</td>
<td>41±1</td>
<td>12±2</td>
<td>287±7</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>10</td>
<td>7.309±39</td>
<td>117±16</td>
<td>366±66</td>
<td>7.40±0.04</td>
<td>91±7</td>
<td>39±4</td>
<td>136±3</td>
<td>3.9±0.2</td>
<td>42±2</td>
<td>32±4*</td>
<td>312±9*</td>
</tr>
</tbody>
</table>

Values are mean±SD. MABP, mean arterial blood pressure. Hct, hematocrit. *p<0.01 different from normoglycemic rats with same hematocrit using analysis of variance and t test.

Discussion

The rCBF has been shown to decrease during chronic hyperglycemia,3-5 a condition accompanied by increased hematocrit in rats.3 If the decrease in rCBF is due to an elevation in hematocrit, normalization of the hematocrit by hemodilution would prevent the decrease. My data indicate that rCBF is reduced in chronically hyperglycemic rats despite normalization of the hematocrit by hemodilution. Unexpectedly, chronic hyperglycemia was not accompanied by increased hematocrit in this study. However, rCBF was also reduced in hyperglycemic rats undergoing sham hemodilution. Both observations indicate that the decreased rCBF during hyperglycemia is not explained by an elevated hematocrit.

Regional variations in the effect of chronic hyperglycemia on rCBF have been noted previously.3 Based on this previous observation, an arbitrary anatomic distinction was made, separating telencephalic from nontelencephalic brain regions. No effect of plasma glucose concentration was apparent in the telencephalic regions (p=0.29), but such an effect was present in the nontelencephalic regions (p=0.002), where mean rCBF reduction was 17%, which is consistent with previous reports.2 3 The
cause of this regional variation in rCBF response remains unexplained. However, rostral-caudal gradients in the rCBF response observed in neonatal dogs during asphyxia have been ascribed to the known density gradient of adrenergic innervation of the cerebral blood vessels. Recently, the attenuation of cerebral hyperemia by indomethacin during seizures was reported to be more prominent in the diencephalon-mesencephalon, pons, and medulla of piglets. Although the cause of reduced rCBF during hyperglycemia is not known, the presence of regional variations in blood flow control must be recognized.

Hemodilution increased global CBF in both normoglycemic and hyperglycemic rats. A hematocrit decrease of 6 units (13%, from 47% to 41%) increased rCBF by 14%. This hematocrit span includes the normal hematocrit for rats, which in our laboratory is 44%. Although there are few studies of the effects of hematocrit on rCBF in rats, the magnitude of this change in rCBF is similar to that reported for dogs, lambs, and humans. It has been noted that hematocrit changes of <15% do not produce detectable changes in CBF in humans. The rCBF increase that I measured occurred after a 13% decrease in hematocrit, indicating that hematocrit should be considered a relevant parameter in studies of rCBF in rats.

CBF varies inversely with hematocrit because of the contribution of erythrocytes to brain metabolism and blood rheology. rCBF is regulated, in part, by the ability of blood to supply oxygen to the tissue, as evidenced by the inverse relation of CBF to blood oxygen content and the direct relation of CBF to oxyhemoglobin affinity. These factors are defined by the structure and amount of hemoglobin in the erythrocytes and are thereby indirectly influenced by acute changes in hematocrit. Erythrocyte concentration directly influences whole blood viscosity. Although the contributions of the rheologic and metabolic influences of changing hematocrit on rCBF may vary within the range over which hematocrit changes, at near-normal hematocrits their contributions appear to be roughly equal.

It is conceivable that chronic hyperglycemia alters the usual relation between erythrocyte concentration and rCBF, producing a lower rCBF at a given hematocrit. Chronic hyperglycemia induced by streptozotocin could increase erythrocyte rigidity and adhesiveness. These factors would reduce rCBF by increasing whole blood viscosity. Chronic hyperglycemia increases oxyhemoglobin affinity and glycosylates hemoglobin. Increased amounts of defective hemoglobin with increased oxygen affinity would act to increase rCBF. The relative contributions of these opposing factors to total cerebrovascular resistance are unknown. However, these influences would act equally on all brain regions. The presence of regional variations in the rCBF reduction measured during chronic hyperglycemia contrasted with the global response to hemodilution is evidence that these factors do not explain the rCBF reduction.

Chronic hyperglycemia could increase the concentrations of plasma components that contribute to blood viscosity, such as fibrinogen and α-2-macroglobulin, and lower rCBF by a viscosity-mediated mechanism that is independent of the hematocrit. Although this possibility cannot be excluded by my data, plasma fibrinogen concentration decreases during streptozotocin-induced hyperglycemia (unpublished data), making this possibility less likely. Again,
the presence of regional variations in the reduction of rCBF during chronic hyperglycemia argues against a viscosity-mediated mechanism for the reduction in rCBF. My data indicate that erythrocyte concentration cannot explain the reduction in rCBF during chronic hyperglycemia and that the effects of hyperglycemia and hemodilution on CBF are independent.

Acknowledgments

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References


Key Words: hematocrit • hyperglycemia • cerebral blood flow • rats
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