Triple-Tracer Autoradiography Demonstrates Effects of Hyperglycemia on Cerebral Blood Flow, pH, and Glucose Utilization in Cerebral Ischemia of Rats

Hirofumi Nakai, MD, Y. Lucas Yamamoto, MD, PhD, Mirko Diksic, PhD, K.J. Worsley, PhD, and Eiichi Takara, MD

Triple-tracer autoradiography was used to measure topographic changes in local cerebral blood flow, cerebral tissue pH, and local cerebral glucose utilization in hyperglycemic and normoglycemic rats, all of which had undergone occlusion of the middle cerebral artery. More severe and extensive reduction of all three variables was observed in the hyperglycemic than in the normoglycemic rats. In seven normoglycemic rats, significant reduction in local cerebral blood flow (p < 0.025) was observed in the ischemic but not in the contralateral nonischemic side at the lateral portion of the caudate nucleus and the neocortex. Tissue pH was significantly lower (p < 0.025) only at the lateral portion of the caudate nucleus in the ischemic side. No significant differences in local cerebral glucose utilization were observed when the two hemispheres were compared. In the ischemic hemisphere of five hyperglycemic rats, the caudate nucleus and the neocortex exhibited significant reduction (p < 0.025) in local cerebral blood flow, tissue pH, and local cerebral glucose utilization. Even in the nonischemic hemisphere of the hyperglycemic rats, local cerebral blood flow in the caudate nucleus and the neocortex was significantly reduced (p < 0.025) compared with the normoglycemic rats. No significant change in tissue pH or local cerebral glucose utilization was observed throughout the nonischemic hemisphere of the hyperglycemic compared with the normoglycemic rats. Tissue pH was systematically lower in the hyperglycemic than in the normoglycemic rats. The threshold level of local cerebral blood flow for tissue pH reduction was 49 ml/100 g/min with a 95% confidence interval of 46–54 ml/100 g/min in normoglycemic rats (37% of control) and 63 ml/100 g/min with a 95% confidence interval of 58–70 ml/100 g/min in hyperglycemic rats (47% of control). The threshold level of local cerebral blood flow for local cerebral glucose hypermetabolism was 20 ml/100 g/min with a 95% confidence interval of 20–24 ml/100 g/min in normoglycemic rats (15% of control) and 30 ml/100 g/min with a 95% confidence interval of 30–49 ml/100 g/min in hyperglycemic rats (22% of control). (Stroke 1988;19:764–772)

Brain glucose concentration in the early stage of cerebral hypoxia-ischemia may be the determining factor governing the severity of ischemic brain damage. Several clinical reports have indicated that the effect of stroke in hyperglycemic patients is more serious than in normoglycemic patients.1,4 Furthermore, animals made hyperglycemic before cerebral ischemia suffered greater neurologic deficits and more severe morphologic brain damage than those with a normal glucose level.5–8 Experimental studies have suggested that a great increase in the brain’s production of lactic acid during hypoxia-ischemia in glucose-fed animals may be a major damaging agent.9–12 Brain tissue pH changes were correlated with the severity of anaerobic glycolysis indicated by mismatching between local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCGU).11,13,14 The reduction of pH in the intracellular and extracellular spaces associated with anoxia/hypoxia and ischemia has been known for several decades.15–24 Recently, several researchers using microelectrode techniques have observed that reduction of pH in the extracellular space is closely related to local changes of cerebral blood flow.17,22–24 We recently developed a triple-tracer autoradiographic method25 to obtain, simultaneously, three independent autoradiograms for LCBF, tissue pH, and LCGU in the same histologic section. Elimination of animal-to-animal variability when estimating the relations between LCBF, tissue pH, and LCGU is the major advantage of our method.25 We compare the relations between LCBF and tissue pH or LCGU in ischemic brain tissue in normoglycemic and hyperglycemic rats following occlusion of the middle cerebral artery (MCA) as measured with our recently developed method of triple-tracer autoradiography.25

From the Cone Neurosurgical Research Laboratory and Neuroisotope Laboratory, Montreal Neurological Institute (H.N., Y.L.Y., M.D., E.T.) and the Department of Mathematics and Statistics (K.J.W.), McGill University, Montreal, Canada. H.N. was on leave from the Department of Neurosurgery, Asahikawa Medical College, Hokkaido, Japan.


Supported in part by the Medical Research Council of Canada (MT-3174) and the Killam Scholarship Fund of the Montreal Neurological Institute.

Address for reprints: Dr. Y.L. Yamamoto or Dr. M. Diksic, Neuroisotope Laboratory, Montreal Neurological Institute, 3801 University Street, Room 636, Montreal, Quebec, Canada H3A 2B4.

Received May 8, 1987; accepted December 14, 1987.
Materials and Methods

Quantitative Triple-Tracer Autoradiographic Technique

The triple-tracer autoradiographic method for measuring LCBF, tissue pH, and LCGU using [18F]fluorodopa, [14C]dimethylxaloxide-2,5-dione, and [3H]deoxyglucose, respectively, as tracers is described in Nakai et al. Autoradiographic differentiation of radioactivity for the three radioisotopes was performed by combining different physical half-lives and energies of isotopes and then removing one tracer from the brain sections during incubation in an organic solvent. The tracers used are [18F] (t1/2 = 110 min, Emax = 240 KeV), [14C] (t1/2 = 5730 yr, Emax = 45 KeV), and [3H] (t1/2 = 12.3 yr, Emax = 5.7 KeV).

General Procedures

Twelve male Sprague-Dawley rats weighing 280–320 g were used. They were anesthetized with 1.5–2.0% halothane with topical application of 2% lidocaine jelly to all wound sites during cannulation of the femoral vessels and occlusion of the MCA. The rats were allowed to awaken from anesthesia, and the lower half of the body was immobilized with a loose-fitting plaster cast on a lead block. Body temperature was kept at approximately 37°C with a heating pad. Blood pressure and blood gases were serially checked during the experiments. Five hyperglycemic rats were fed Rat Chow 5012 (Purina Mills Inc., St. Louis, Missouri), which contains 22% protein, 4% fat, and 5% carbohydrate, ad libitum until anesthesia. Fed rats were used for the hyperglycemic protocol because of the ease with which we were able to obtain a stable hyperglycemic state for several hours. Mean ± SD glucose concentration of arterial plasma in the hyperglycemic rats was 315 ± 13 mg/dl for the entire experiment, almost double that in the seven normoglycemic rats fasted, except for water, for 18 hours (167 ± 29 mg/dl). Rats and triple-tracer autoradiograms were prepared as described in Nakai et al.

Statistical Analysis

LCBF threshold for tissue pH reduction. The relation between tissue pH and the LCBF threshold T, below which pH became unstable, was estimated by the following method. pH was assumed to be normally distributed, with constant SD and a mean m that was constant for LCBF > T and that decreased linearly with a slope b for LCBF < T. For a given T, the parameters m and b can be estimated from simple linear regression of pH on a single variable, taking the minimum values LCBF - T and 0. The maximum-likelihood estimate of T can be obtained by choosing the value that minimizes the error sum of squares (SSE). The method of Hinkley was used to find an approximate confidence interval (CI) for T, and the method of Worsley was used to test for a decrease in pH for the hyperglycemic rats with LCBF > T estimated for the normoglycemic rats.

Results

Physiological Variables

Physiological variables for both groups of rats are given in Table 1. There was no significant difference between groups except for the arterial plasma concentration of glucose.

Simultaneous Measurements of LCBF, Tissue pH, and LCGU

Normoglycemic rats. Figure 1 shows a typical set of [18F]fluorodopa, [14C]dimethylxaloxide-2,5-dione, and [3H]deoxyglucose autoradiograms of selected brain cross sections in a normoglycemic rat obtained by triple-tracer autoradiography 3 hours after MCA occlusion. Ten anatomic structures close to the MCA territory were quantitatively evaluated (Table 2). LCBF in all except two structures (globus pallidus and thalamus) decreased significantly (p < 0.025) compared with homologous structures in the contralateral nonischemic side. Although all 10 structures in the ischemic side showed generally decreased tissue pH, there was a significant decrease (p < 0.025) only in the lateral portion of the caudate. LCGU was lower in all structures in the ischemic side; however, the difference

<table>
<thead>
<tr>
<th>Table 1. Physiological Variables: Triple-Tracer Autoradiography in Normoglycemic and Hyperglycemic Rats With Middle Cerebral Artery Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Mean arterial blood pressure</td>
</tr>
<tr>
<td>(mm Hg)</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Arterial plasma glucose</td>
</tr>
<tr>
<td>concentration (mg/dl)</td>
</tr>
<tr>
<td>Hematocrit</td>
</tr>
<tr>
<td>Before experiment (%)</td>
</tr>
<tr>
<td>At end of experiment (%)</td>
</tr>
<tr>
<td>Values are mean ± SD. Mean arterial blood pressure, Paco2,</td>
</tr>
<tr>
<td>Values are mean ± SD. Mean arterial blood pressure, Paco2,</td>
</tr>
</tbody>
</table>
was not significant. Regional changes are depicted in Figure 2 and quantified in Table 3.

**Hyperglycemic rats.** Figure 3 shows a typical set of ^18^F-FAP, ^14^C-DMO, and ^2^H-2-DG autoradiograms of selected brain cross sections in a hyperglycemic rat; LCBF, pH, and LCGU are quantified in Table 2. Regional changes are depicted in Figure 4 and quantified in Table 3.

**Comparison.** Comparison of the ischemic sides of normoglycemic and hyperglycemic rats showed a significant decrease (p < 0.025) of tissue pH and LCGU in the neocortex and the caudate and of LCBF in the neocortex of the hyperglycemic rats (Table 2). Furthermore, a significant decrease (p < 0.025) in LCBF was observed in the neocortex, the caudate, and the thalamus in the nonischemic sides of hyperglycemic compared with normoglycemic rats. LCGU was systematically lower in all structures in the nonischemic sides of hyperglycemic rats compared with that in normoglycemic rats, but the difference was not significant. Tissue pH in the nonischemic sides was, however, not different in the two groups. Since there is no data on the lumped and rate constants for deoxyglucose during the acute state of ischemia, the lumped and rate constants measured in normal rat brain were used to calculate the LCGU in ischemic and hyperglycemic brain.** Comparison of autoradiograms at the caudate level clearly revealed much more severe and extensive regional changes of LCBF, tissue pH, and LCGU in hyperglycemic (Figure 4, Table 3) than in normoglycemic rats (Figure 2, Table 3).

**Cerebral Blood Flow Threshold for pH Reduction**

Representative triple-tracer autoradiograms obtained with ^18^F-FAP, ^14^C-DMO, and ^2^H-2-DG in normoglycemic and hyperglycemic rats are shown in...
Nonischemic hemisphere

Ischemic hemisphere

Occlusion of Left Middle Cerebral Artery in Rats

Assumed to be 7.5% instead of 15%.

Utilization. When calculating tissue pH in selected anatomic structures (except for globus pallidus and thalamus), % extracellular space was assumed to be 7.5% instead of 15%.

Values in the ischemic sides of normoglycemia and hyperglycemia rats were plotted as a function of LCBF (Figures 5-7).

Estimated mean pH when LCBF was >49 ml/100 g/min. The same model with a linear decrease in pH from an unknown LCBF was fitted to the data were analyzed only in the range of stable normoglycemic LCBF; that is, an LCBF of ≥50 ml/100 g/min. The same model with a linear decrease in pH from an unknown LCBF < T was fitted to the

Table 2. Normoglycemia vs. Hyperglycemia: LCBF, Tissue pH, and LCGU in Selected Anatomic Structures of Brain After 3-Hour Occlusion of Left Middle Cerebral Artery in Rats

<table>
<thead>
<tr>
<th>Structure</th>
<th>LCBF (ml/100 g/min)</th>
<th>Tissue pH</th>
<th>LCGU (μmol/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normoglycemia</td>
<td>Hyperglycemia</td>
<td>Normoglycemia</td>
</tr>
<tr>
<td><strong>Ischemic hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>47±23*</td>
<td>27±10</td>
<td>6.90±0.05</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>38±21*</td>
<td>18±5†</td>
<td>6.86±0.09</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>38±17*</td>
<td>17±4†</td>
<td>6.87±0.04</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>46±19*</td>
<td>17±7†‡</td>
<td>6.87±0.07</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>73±28*</td>
<td>45±17</td>
<td>6.88±0.05</td>
</tr>
<tr>
<td><strong>Caudate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral</td>
<td>20±20*</td>
<td>14±20</td>
<td>6.78±0.18*</td>
</tr>
<tr>
<td>Medial</td>
<td>52±34*</td>
<td>30±22</td>
<td>6.86±0.19</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>49±12</td>
<td>40±12</td>
<td>6.89±0.06</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral nucleus</td>
<td>95±30</td>
<td>83±38</td>
<td>6.93±0.05</td>
</tr>
<tr>
<td>Medial</td>
<td>80±28</td>
<td>73±27</td>
<td>6.92±0.05</td>
</tr>
<tr>
<td><strong>Nonischemic hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>125±9</td>
<td>108±9†</td>
<td>6.94±0.03</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>131±13</td>
<td>103±12†‡</td>
<td>6.93±0.03</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>145±34</td>
<td>96±10†</td>
<td>6.90±0.05</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>175±38</td>
<td>121±31†‡</td>
<td>6.92±0.03</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>106±27</td>
<td>78±15</td>
<td>6.89±0.04</td>
</tr>
<tr>
<td><strong>Caudate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral</td>
<td>103±21</td>
<td>74±15†</td>
<td>6.95±0.01</td>
</tr>
<tr>
<td>Medial</td>
<td>103±23</td>
<td>71±11†</td>
<td>6.96±0.02</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>61±20</td>
<td>49±7</td>
<td>6.92±0.06</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral nucleus</td>
<td>121±17</td>
<td>90±20†‡</td>
<td>6.95±0.05</td>
</tr>
<tr>
<td>Medial</td>
<td>103±13</td>
<td>80±17†</td>
<td>6.93±0.07</td>
</tr>
</tbody>
</table>

Values are mean±SD (n=7 in normoglycemia, n=5 in hyperglycemia). LCBF, local cerebral blood flow; LCGU, local cerebral glucose utilization. When calculating tissue pH in selected anatomic structures (except for globus pallidus and thalamus), % extracellular space was assumed to be 7.5% instead of 15%.

*p<0.025 compared with nonischemic side, one-tailed t test.

†p<0.025 compared with normoglycemia, one-tailed t test.

‡p<0.025 compared with normoglycemia, one-tailed t test using Bonferroni’s multiple comparison procedure.

Figures 2 and 4 and Table 3. Regional pH and LCGU values in the ischemic sides of normoglycemic and hyperglycemic rats were plotted as a function of LCBF (Figures 5–7).

To satisfy the assumption of equal SD of pH, pH data with LCBF of <22 ml/100 g/min were ignored because the pH fluctuation was much greater than that with LCBF of ≥22 ml/100 g/min even though mean pH continued to decrease. Estimated T for normoglycemic rats was 49 ml/100 g/min, with a 95% Cl of 46–54 ml/100 g/min (Figures 5 and 7). Estimated mean pH when LCBF was >49 ml/100 g/min was $m = 6.942 \pm 0.005$, changing with a slope $b = 0.0062 \pm 0.0005$ when LCBF was <49 ml/100 g/min.

To detect a change in pH for hyperglycemic rats, the data were analyzed only in the range of stable normoglycemic LCBF; that is, an LCBF of ≥50 ml/100 g/min. The same model with a linear decrease in pH from an unknown LCBF < T was fitted to the

Table 3. Regional Changes in LCBF, Tissue pH, and LCGU After 3-Hour Occlusion of Left Middle Cerebral Artery in Rats

<table>
<thead>
<tr>
<th>Region</th>
<th>LCBF</th>
<th>Tissue pH</th>
<th>LCGU</th>
<th>LCBF</th>
<th>Tissue pH</th>
<th>LCGU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemia</td>
<td></td>
<td></td>
<td></td>
<td>Hyperglycemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>127</td>
<td>6.90</td>
<td>96</td>
<td>112</td>
<td>6.92</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>6.82</td>
<td>59</td>
<td>9</td>
<td>6.18</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>6.74</td>
<td>64</td>
<td>9</td>
<td>6.24</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>6.36</td>
<td>114</td>
<td>6</td>
<td>6.28</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>6.20</td>
<td>97</td>
<td>4</td>
<td>5.76</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>6.60</td>
<td>35</td>
<td>4</td>
<td>5.54</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>6.70</td>
<td>45</td>
<td>2</td>
<td>7.09</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>6.50</td>
<td>49</td>
<td>1</td>
<td>8.16</td>
<td>3</td>
</tr>
</tbody>
</table>
hyperglycemia data with the constraint that stable pH was fixed at \( m = 6.94 \), the median stable normoglycemic pH. The estimated point of inflection was at \( T = 63 \text{ ml/100 g/min} \), with an approximate 95% CI of 58–70 ml/100 g/min (Figures 6 and 7). The estimated slope was \( b = 0.0047 \pm 0.0012 \), slightly lower than that in normoglycemia. A conservative test of the significance of this difference in slope was obtained by applying a Bonferroni correction to the \( t \) statistic \( t = 3.98 \) (15 df), as suggested by Worsley. The result was significant at \( p < 0.01 \), though the results of Worsley indicate that the actual \( p \) value may be half this figure. This is strong evidence that as LCBF decreases, pH in hyperglycemic rats decreases at a greater LCBF than in normoglycemic rats (Figure 7). Below LCBF of 50 ml/100 g/min, the pH of hyperglycemic rats decreases at a much greater rate of \( b = 0.0163 \pm 0.0032 \).

Cerebral Blood Flow Thresholds for Hypermetabolism of Glucose

For normoglycemic rats, the estimated \( T \) was 20 ml/100 g/min, with a 95% CI of 20–24 ml/100 g/min (Figure 5). For hyperglycemic rats, the estimated \( T \) was 30 ml/100 g/min, with a 95% CI of 30–49 ml/100 g/min (Figure 6). There is no overlap in CIs, indicating that hypermetabolism of LCGU occurs in hyperglycemia at a much greater LCBF than in normoglycemia. The method of Worsley was used to test for an increase in the probability \( p \) for the hyperglycemia data, with LCBF in the normal range for normoglycemia, that is, with LCBF of >30 ml/100 g/min. The result was highly significant \( (p < 0.00005) \).

Discussion

Simultaneous measurement of LCBF, tissue pH, and LCGU by triple-tracer autoradiography is of particular

![Figure 3. Triple-tracer autoradiograms after 3-hour occlusion of left middle cerebral artery in hyperglycemic rat, using 4-[^14]F]fluoroantipyrine for local cerebral blood flow (LCBF) (A–C), [1^14]C]dimethylazodiol-2,5-dione for tissue pH (D–F), and 2-[^3]H]deoxyglucose for local cerebral glucose utilization (LCGU) (G–I). Variable reduction of LCBF is shown (A–C). In mildly ischemic area, pattern of reduction is similar in LCGU, but there is not as much change in tissue pH as in LCBF. However, in moderately to severely ischemic area, heterogeneous change of LCGU (mixture of severe increase and decrease) and extremely severe decrease of tissue pH occur. Severely decreased tissue pH increases in both degree and extent in hyperglycemic rat.](http://stroke.ahajournals.org/)

![Figure 4. Representative triple-tracer autoradiograms of (A) ^14]F-FAP, (B) ^14]C-DMO, and (C) ^3]H-2-DG after 3-hour occlusion of left middle cerebral artery in hyperglycemic rat. Regional change of local cerebral blood flow, tissue pH, and local cerebral glucose utilization is shown in Table 3.](http://stroke.ahajournals.org/)
FIGURE 5. Normoglycemia. Top: Individual cerebral tissue pH values obtained from ischemic hemisphere after 3-hour occlusion of left middle cerebral artery (MCA) in normoglycemic rat plotted in relation to local cerebral blood flow (LCBF). Shaded area represents normal range of tissue pH obtained from mean ±3 SD of contralateral nonischemic hemisphere tissue pH. Bottom: Relation between LCBF and local cerebral glucose utilization (LCGU) after 3-hour occlusion of left MCA in normoglycemic rat. LCGU shows bimodal response to decreasing LCBF, that is, LCGU decreases with reduction of LCBF until LCBF is reduced to 20 ml/100 g/min. Below this level, LCGU increases sharply used to calculate the values of these variables. Several assumptions were made in the calculations. We used the partition coefficient of normal brain to calculate LCBF and water content of normal brain. We estimated the percent extracellular space in calculating tissue pH, and we used the lumped and rate constants of normal brain in calculating LCGU. These assumptions may produce some errors when calculating absolute values of respective pathophysiologic variables. Despite these assumptions, we believe that our results are informative because the simultaneous measurement of these variables greatly facilitates interpretation of the result obtained. Since our data excludes animal-to-animal variability from the interrelations of different variables, the interpretation is greatly enhanced compared with previous reports.

FIGURE 6. Hyperglycemia. Top: Individual cerebral tissue pH values obtained from ischemic hemisphere after 3-hour occlusion of left middle cerebral artery (MCA) in hyperglycemic rat plotted in relation to local cerebral blood flow (LCBF). Shaded area represents normal range of tissue pH obtained from mean ±3 SD of contralateral nonischemic hemisphere tissue pH. Bottom: Relation between LCBF and local cerebral glucose utilization (LCGU) after 3-hour occlusion of left MCA in hyperglycemic rat. LCGU shows bimodal response to decreasing LCBF, that is, LCGU decreases with reduction of LCBF until LCBF is reduced to 30 ml/100 g/min. Below this level, LCGU increases sharply, with one region still showing LCGU decreased to some extent.

interest for the study of pathologic processes that are difficult to standardize. Particularly in the MCA occlusion model, a large variation in extent, degree, and location of the ischemia, as indicated by previous reports, necessitated simultaneous measurements of these variables. It should be noted that in pathologic conditions, triple-tracer autoradiography has the same limitations as single-tracer autoradiography in regard to the validity of the biologic models...
The evoked potential starts to decrease at 20 ml/100 g/min, with biphasic slopes of 0.0047 ± 0.0012 and 0.0062 ± 0.0005. In hyperglycemic rats, LCBF threshold is 63 ml/100 g/min, with biphasic slopes of 0.0047 ± 0.0012 and 0.0163 ± 0.0032.

In our study, fed and fasted rats were used to obtain hyperglycemic and normoglycemic conditions. Pulsinelli et al. noted that plasma glucose concentration two to three times that in normoglycemic rats was sufficient to induce more severe neuropathologic brain damage following cerebral ischemia. Some recent reports have claimed that brain glucose concentration at the early stage of cerebral ischemia is one of the important prognostic factors determining the outcome of cerebral ischemia. Studies in experimental animals made hyperglycemic before cerebral ischemia showed that they suffered greater neurologic deficits and more severe morphologic brain damage than did normoglycemic animals.

The concept of thresholds of ischemia has been developed, relating various types of functional disturbances to well-defined levels of remaining blood flow. The evoked potential starts to decrease at 20 ml/100 g/min, and evoked potentials are completely suppressed at < 15 ml/100 g/min. The thresholds of ion pump failure are 6–8 ml/100 g/min. These thresholds are remarkably similar in different species and under different anesthetics, indicating that basic mechanisms are involved.

Our data on the correlation of LCBF, tissue pH, and LCGU using triple-tracer autoradiography indicate that the LCBF threshold for tissue pH reduction in normoglycemic rats is 49 ml/100 g/min, with a 95% CI of 46–54 ml/100 g/min, and the LCBF threshold for LCGU hypermetabolism is 20 ml/100 g/min, with a 95% CI of 20–24 ml/100 g/min. That the LCBF threshold level for tissue pH reduction is certainly much higher than that of electrical function failure confirms previous extracellular microelectrode findings in which the reduction of extracellular pH appeared at much higher cerebral blood flows than the extracellular potassium ion changes.

Harris and Symon have shown that in ischemic tissue in rats, the extracellular pH falls steadily at LCBF of < 30 ml/100 g/min. (The ischemia was caused by bilateral carotid artery occlusion with progressive hypotension produced by gradual bleeding.) These researchers measured LCBF using a microelectrode technique in the extracellular spaces of the cortex in the area of the MCA. Normal LCBF in the cortex was 79 ± 28 ml/100 g/min. Thus, in their study the LCBF threshold for pH reduction, 30 ml/100 g/min, was 38% of normal cortical LCBF values. More recently, Harris et al. have shown that the extracellular pH fell steadily when LCBF was < 20 ml/100 g/min in progressive ischemia of the primate cerebral cortex. They also measured LCBF by the microelectrode method. Control LCBF was 58 ± 15 ml/100 g/min. Thus, the LCBF threshold for pH reduction was 34% of control values in baboons. In our studies in which LCBF was measured by quantitative autoradiography, normal cortical LCBF in the area of the MCA in awake rats was 134 ± 10 ml/100 g/min. Thus, the LCBF threshold for pH reduction, 49 ml/100 g/min, was 37% of control cortical LCBF. Therefore, the absolute value of the LCBF threshold for pH reduction differs with blood flow measurement techniques and with species. Although control LCBF varies with these methologic and biologic differences, the LCBF threshold for pH reduction was relatively consistent, between 34% and 38% of control LCBF in both the findings of Harris et al. and of our group. The LCBF threshold should, therefore, be expressed as percent of control LCBF. This would avoid confusion with an absolute value expression, which can vary with LCBF methodologies. Our findings also indicate that LCBF thresholds for both tissue pH reduction and LCGU hypermetabolism are higher in hyperglycemic rats. Furthermore, in hyperglycemic rats, tissue pH reduction is more severe and extensive (Table 2, Figure 4). We have also observed that the LCBF threshold for LCGU hypermetabolism is less sensitive than that of tissue pH reduction.

We have observed a significant reduction (p < 0.025) of LCBF in both the ischemic and nonischemic sides of hyperglycemic compared with normoglycemic rats. These data support the previous observations by Ginsberg et al. and Duckrow et al. Several reasons have been suggested for general reduction of LCBF under hyperglycemic states: increased cerebrovascular resistance caused by plasma hyperosmolality, increased blood viscosity, and decreased cerebral metabolic rate. However, changes in osmolality and viscosity factors might not totally explain the reduction in LCBF in hyperglycemic animals. Reduction of LCGU in the nonischemic side of hyperglycemic rats was reduced only slightly (12 ± 4%) compared with normoglycemic rats. Once an ischemic insult was induced, earlier and more severe pH and metabolic

**FIGURE 7.** Computer graphic display of statistical analysis for local cerebral blood flow (LCBF) thresholds of tissue pH reduction after 3-hour occlusion of left middle cerebral artery in normoglycemic and hyperglycemic rats. In normoglycemia, LCBF threshold is 49 ml/100 g/min, with a slope of 0.0062 ± 0.0005. In hyperglycemia, LCBF threshold is 63 ml/100 g/min, with biphasic slopes of 0.0047 ± 0.0012 and 0.0163 ± 0.0032.

Data from our laboratory measurements in baboons. In our studies in which LCBF was measured by quantitative autoradiography, normal cortical LCBF in the area of the MCA in awake rats was 134 ± 10 ml/100 g/min. Thus, the LCBF threshold for pH reduction, 49 ml/100 g/min, was 37% of control cortical LCBF. Therefore, the absolute value of the LCBF threshold for pH reduction differs with blood flow measurement techniques and with species. Although control LCBF varies with these methologic and biologic differences, the LCBF threshold for pH reduction was relatively consistent, between 34% and 38% of control LCBF in both the findings of Harris et al. and of our group. The LCBF threshold should, therefore, be expressed as percent of control LCBF. This would avoid confusion with an absolute value expression, which can vary with LCBF methodologies. Our findings also indicate that LCBF thresholds for both tissue pH reduction and LCGU hypermetabolism are higher in hyperglycemic rats. Furthermore, in hyperglycemic rats, tissue pH reduction is more severe and extensive (Table 2, Figure 4). We have also observed that the LCBF threshold for LCGU hypermetabolism is less sensitive than that of tissue pH reduction.

We have observed a significant reduction (p < 0.025) of LCBF in both the ischemic and nonischemic sides of hyperglycemic compared with normoglycemic rats. These data support the previous observations by Ginsberg et al. and Duckrow et al. Several reasons have been suggested for general reduction of LCBF under hyperglycemic states: increased cerebrovascular resistance caused by plasma hyperosmolality, increased blood viscosity, and decreased cerebral metabolic rate. However, changes in osmolality and viscosity factors might not totally explain the reduction in LCBF in hyperglycemic animals. Reduction of LCGU in the nonischemic side of hyperglycemic rats was reduced only slightly (12 ± 4%) compared with normoglycemic rats. Once an ischemic insult was induced, earlier and more severe pH and metabolic

**FIGURE 7.** Computer graphic display of statistical analysis for local cerebral blood flow (LCBF) thresholds of tissue pH reduction after 3-hour occlusion of left middle cerebral artery in normoglycemic and hyperglycemic rats. In normoglycemia, LCBF threshold is 49 ml/100 g/min, with a slope of 0.0062 ± 0.0005. In hyperglycemia, LCBF threshold is 63 ml/100 g/min, with biphasic slopes of 0.0047 ± 0.0012 and 0.0163 ± 0.0032.
changes followed in the ischemic side of hyperglycemic than of normoglycemic rats. Therefore, secondary events associated with cerebral ischemia, such as development of cerebral edema, release of vasoactive substances, loss of autoregulation, and diaschisis, might also occur more rapidly in hyperglycemic animals following ischemia. Significant reduction of LCBF in the ischemic cerebral hemisphere of hyperglycemic compared with normoglycemic animals might be caused by a combination of these secondary events.

Acknowledgments

We would like to express our thanks to the staff of the Cone Laboratory for Neuroresearchal Research and of the Medical Cyclotron for their assistance, and to Ms. F. Lumia for typing this manuscript.

References

27. Worsley KJ: The power of likelihood ratio and cumulative sum tests for a change in a binomial probability. Biometrika 1983;70:455–464

Nakai et al Triple-Trace Autoradiography 771

Downloaded from http://stroke.ahajournals.org/ by guest on October 22, 2017
47. Duckrow RB, Beard DC, Brennan RW: Regional cerebral blood flow decreases during chronic and acute hyperglycemia. Stroke 1987;18:52–58

Key Words • autoradiography • cerebral blood flow • cerebral ischemia • hyperglycemia • rats
Triple-tracer autoradiography demonstrates effects of hyperglycemia on cerebral blood flow, pH, and glucose utilization in cerebral ischemia of rats.

H Nakai, Y L Yamamoto, M Diksic, K J Worsley and E Takara

Stroke. 1988;19:764-772
doi: 10.1161/01.STR.19.6.764

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/19/6/764

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/