Effects of Glucose and PaO₂ Modulation on Cortical Intracellular Acidosis, NADH Redox State, and Infarction in the Ischemic Penumbra

Robert E. Anderson, BS; William K. Tan, PhD; Heidi S. Martin; Fredric B. Meyer, MD

Background and Purpose—During focal cerebral ischemia, the ischemic penumbra or border-zone regions of moderate cortical blood flow reductions have a heterogeneous development of intracellular cortical acidosis. This experiment tested the hypotheses that (1) this acidosis is secondary to glucose utilization and (2) this intracellular acidosis leads to recruitment of potentially salvageable tissue into infarction.

Methods—Brain pHᵢ, regional cortical blood flow, and NADH redox state were measured by in vivo fluorescent imaging, and infarct volume was assessed by triphenyltetrazolium chloride histology. Thirty fasted rabbits divided into 6 groups of 5 each were subjected to 4 hours of permanent focal ischemia in the presence of hypoglycemia (≈2.8 mmol/L), moderate hyperglycemia (≈11 mmol/L), and severe hyperglycemia (>28 mmol/L) under either normoxia or moderate hypoxia (PaO₂ ≈50 mm Hg).

Results—Preischemic hyperglycemia led to a more pronounced intracellular acidosis and retardation of NADH regeneration than in the hypoglycemia groups under both normoxia and moderate hypoxia in the ischemic penumbra. For example, 4 hours after ischemia, brain pHᵢ in the severe hyperglycemia/normoxia group measured 6.46, compared with 6.84 in the hypoglycemia/normoxia group (P<0.01), and NADH fluorescence measured 173% compared with 114%. Infarct volume in the severe hyperglycemia/normoxia group measured 35.1±6.9% of total hemispheric volume, compared with 13.5±4.2% in the hypoglycemia/normoxia group (P<0.01).

Conclusions—Hyperglycemia significantly worsened both cortical intracellular brain acidosis and mitochondrial function in the ischemic penumbra. This supports the hypothesis that the evolution of acidosis in the ischemic penumbra is related to glucose utilization. Furthermore, the observation that hypoglycemia significantly decreased infarct size supports the postulate that cortical acidosis leads to recruitment of ischemic penumbra into infarction. (Stroke. 1999;30:160-170.)

Key Words: acidosis ■ redox, NADH ■ cerebral infarction ■ glucose ■ cerebral ischemia, focal ■ rabbits

When the cortical surface is imaged during focal cerebral ischemia, there is variation in the development of intracellular acidosis.1-3 In regions of severe cerebral blood flow reductions or evolving infarction, the decline in intracellular pH (pHᵢ) is relatively uniform and profound. In the border-zone region of moderate ischemia surrounding the evolving infarction, however, there is a heterogeneous distribution of intracellular acidosis. The significance of this intracellular acidosis in the ischemic penumbra is unclear.

The mechanisms by which acidosis contributes to neuronal injury may include facilitating free radical formation, activating pH-dependent endonucleases with DNA fragmentation, or altering intracellular Ca²⁺ regulation.4-10 Despite the probable deleterious effects of acidosis, the effects of hyperglycemia in focal cerebral ischemia remain controversial.11-14 For example, it has been published that hyperglycemia reduces, does not alter, or increases damage after transient focal cerebral ischemia.11-14 It has also been published that hypoglycemia decreases the degree of pan-necrosis.13 It is possible that varying effects of hyperglycemia may be due to differences in collateral blood flow and therefore the degree of lactic acid production.13 The effects of hyperglycemia may also be dependent on reperfusion.16 Adding to the controversy are in vitro observations that acidosis may ameliorate neuronal injury caused by glutamate and anoxia.17,18

This experiment tested the hypotheses that (1) the development of cortical intracellular acidosis in the ischemic penumbra is a result of glucose utilization and (2) this acidosis leads to recruitment of potentially salvageable tissue into infarction. To test this hypothesis, in vivo fluorescence imaging was used to measure brain pHᵢ, regional cortical blood flow (rCBF), and the NADH redox state in the New...
Zealand White rabbit. Histological assessment of infarction was performed in the acute setting with tetrazolium staining.

Materials and Methods

Animal Preparation

After review and approval by the Institutional Animal Care and Use Committee, 30 New Zealand White rabbits weighing between 3.5 and 4.5 kg that had been fasted overnight were induced with sodium pentothal 40 mg/kg, operated on under 2.5% halothane anesthesia, and studied under 1.5% halothane anesthesia, respectively. A tracheotomy was performed, and the animals were placed on a Harvard respirator (Harvard Apparatus). The animals were given 0.15 mg/kg pancuronium bromide (Pavulon; Organon Inc.) subcutaneously to prevent surface oxygenation and to keep the brain moist. Blood loss for the surgical preparation did not exceed 5 mL.

After the surgical preparation, the animal was moved from the operating table and placed on an intravital-type microscope stand. The microscope was focused on an area centered about the sylvian gyrus, with 1.5 cm² of cortex imaged for brain pH, rCBF, operating table and placed on an intravital-type microscope stand. For the surgical preparation did not exceed 5 mL.

In Vivo Video Fluorescent Instrumentation

Instrumentation was designed to perform serial panoramic video imaging of cortical brain pH, and rCBF with umbelliferone fluorescence. The optical characteristics were such that the majority or a portion of the exposed hemisphere, pH, and rCBF could be studied simultaneously through a large cranietomy by varying the degree of magnification. The use of umbelliferone as a noninvasive in vivo technique for measuring brain pH, and CBF has been described previously. Umbelliferone is nontoxic, fat soluble, and freely diffusible across the blood-brain barrier, and it rapidly equilibrates across cell membranes and is uniformly distributed through the cytoplasm and mitochondrial matrix.

Umbelliferone was prepared for injection by dissolving 0.2 g of indicator in 200 mL of 5% glucose-saline solution at 90°C for 30 minutes. The solution was then filtered through a 0.22-mesh filter before injection. For each measurement, 1.0 mL of umbelliferone was injected retrogradely through a catheter placed in the right lingual artery. The measurements were separated by 30-minute intervals to allow for sufficient clearance of the indicator out of the brain tissue.

The pH-sensitive indicator umbelliferone has 2 fluorophores, anionic and isobestic. The anionic and isobestic forms are excited at 370 and 340 nm, respectively, and have a common emission at 450 nm. The fluorescence of the anion varies directly with pH, whereas the fluorescence of the isobestic form varies directly only with the indicator concentration. Therefore, it is possible to create a nomogram from the ratio of 340- to 370-nm excitations to determine brain pH. NADH fluorescent images were acquired before umbelliferone was injected into the lingual artery for correction of background fluorescence. Intrinsic NADH fluorescence images excited at 370 nm were stored for later analysis of mitochondrial function. The scale factor for the percent change in NADH fluorescence from baseline is set so that at 100%, the level represents the level of NADH fluorescence in normal brain, whereas an increase to 300% represents brain death. The scale factor is confirmed by random measurement of NADH fluorescence levels at death and comparing it with baseline nonischemic values in the same animal. It is important to note that a primary source of artifact in the measurement of NADH fluorescence is hemoglobin interference. It has been shown previously that use of bright-field illumination, as used in this optical system to reduce scatter, minimizes the effect of this type of interference. In a monkey model, correlated biopsy analysis of NADH and NADPH with that of NADH fluorescence measurements in normal brain and brain at death. Use of both techniques showed that there was a close relationship in the degree of change between normal brain and brain at death. The images from the 340-nm excitation were processed to compute rCBF from the 1-minute initial slope index with a partition coefficient of unity for umbelliferone. The rCBF image was then displayed and stored on tape for final analysis. For final analysis, 4-mm coronal sections yielding slices (Figure 1) that represented 2 anterior locations, 2 central locations, and 2 posterior locations of the exposed hemisphere, pH, and rCBF could be studied simultaneously through a large cranietomy by varying the degree of magnification.

Histological Analysis

It was determined by the investigators and consulting veterinarian that permitting the animals to survive for several days to allow for infarct maturation would not be acceptable under current institutional animal care guidelines, given analgesic expectations. Recognizing these limitations, the animals were killed at the end of the ischemic period for acute histological assessment, which did not allow for infarct maturation. At the end of each experiment, the brain was removed. To increase the firmness of the brain for sectioning, it was immersed in saline and chilled for 30 minutes. The brain was then sliced into 4-mm coronal sections, yielding slices (Figure 1) that represented 2 anterior locations, 2 central locations, and 2 posterior locations of the exposed hemisphere, pH, and rCBF could be studied simultaneously through a large cranietomy by varying the degree of magnification.
locations. The sections were then immersed in a 37°C solution of 2%
2,3,5-triphenyltetrazolium chloride (TTC) in saline. To enhance TTC
penetration, the sections were suspended within the TTC solution for
30 minutes in a shaker bath maintained at 37°C. Sections were
removed from the TTC solution, placed flat on one another in
separate fixation cassettes, and stored in 10% buffered formalin.
Photographic slides of each section were taken 1 week later. For
assessment of the amount of tissue damage, each section was
photographed, and the area of infarction was identified, traced, and
digitized. The total area of the hemisphere was also determined.

Definition of Ischemic Penumbra
The purpose of this experiment was to determine the effects of
oxygen and glucose manipulations on brain pH_i and NADH redox
state in the ischemic penumbra. Determination of the location of
the ischemic penumbra was made as follows. Thirty minutes after the
onset of ischemia, in the immediate proximal distribution of the
MCA along the sylvian fissure, there is a relatively small region of
cortex in which rCBF declines to
\[ \text{rCBF} \leq 12 \text{ mL·min}^{-1} \cdot 100 \text{ g}^{-1} \], or an
\( \approx 80\% \) reduction in rCBF values compared with preischemic mea-
surements. Under normoglycemic conditions, this cortex has pH_i
reductions to \( \approx 6.60 \). This region has previously been demonstrated
to be an evolving infarction with necrosis under light microscopic
examination.\(^{19}\) Distal to this zone of severe rCBF reductions is
parietal cortex exposed by the craniectomy that has initial rCBF
reductions of \( \approx 20 \text{ mL·min}^{-1} \cdot 100 \text{ g}^{-1} \). This cortex was defined as
the ischemic penumbra in this experiment, and the tissue was
analyzed by fluorescence imaging and histology. For example,
Figures 2 and 3 are composite images of this cortex, which is distal
to the smaller zone of early evolving infarction. Within this ischemic
penumbra, there is a heterogeneous development of acidosis, which
is the issue of interest for this experiment.

Statistical Analysis
Separate analyses were carried out for each of the 4 variables under
study: pH_i, rCBF, NADH fluorescence, and infarct volume. ANOVA
was used to test the statistical significance of differences between
groups. Results were considered statistically significant at a value of
\( P < 0.05 \). Data are presented as mean±SEM. All analysis was
conducted with CSS (Statsoft) statistical software.

Results
Systemic Parameters, Nonischemic Control Group,
and Video Acquisition
There were no significant differences over time between animals
studied in each of the 6 groups in the measurements of \( \text{Paco}_2 \),
\( \text{Pao}_2 \), pH, mean arterial blood pressure, body temperature,
glucose, and hematocrit (Table). The control sham-operated
nonischemic animals had stable pH_i, rCBF, and NADH fluores-
cence measurements throughout the experiment, thus confirm-
ing the stability of the preparation. The blood serum levels of
\( \text{Pao}_2 \), \%ScO_2, and lactate in the moderate hypoxia groups were
significantly different from those of the normoxia groups
\( (P < 0.01) \). Figures 2 and 3 show composite video pictures of 2
typical experiments: a hypoglycemic/moderately hypoxic animal and a hyperglycemic/normoxic animal, respectively.

**Experimental Groups (Figure 4)**

**Normoxic Groups**

*Brain pH*. Baseline preischemic brain pH was uniform over the exposed cortex in all groups, measuring 6.96±0.03. Within the ischemic penumbra, there was a heterogeneous development of acidosis, as illustrated in an example experiment, Figure 3. After 2 hours of ischemia, overall brain pH in the ischemic penumbra declined to 6.65±0.04 and 6.56±0.10 in the moderate and severe hyperglycemia groups, respectively (P<0.01 compared with preischemic values). In the hypoglycemia group, brain pH declined to 6.88±0.03, which was not significantly different from preischemic values. After 4 hours of ischemia, brain pH declined further, to 6.47±0.06 in both the moderate and severe hyperglycemia groups (P<0.01). In the hypoglycemia group, brain pH was 6.84±0.09, not significantly different from preischemic values. Figure 5 depicts a series of brain pH histograms of a hyperglycemic/normoxic animal (Figure 3), demonstrating that before occlusion, brain pH was relatively homogeneous and then became markedly heterogeneous after the onset of focal ischemia.

*Regional Cortical Blood Flow*. rCBF in all groups was 51.5±3.3 mL·100 g⁻¹·min⁻¹ before ischemia. After 2 hours of ischemia, rCBF in the ischemic penumbra fell significantly (P<0.01) in all groups studied, to 15 mL·100 g⁻¹·min⁻¹. After 4 hours of ischemia, rCBF declined further to 10 mL·100 g⁻¹·min⁻¹ in all groups (P<0.01). Figure 5 depicts a series of rCBF histograms of a hyperglycemic/normoxic animal (Figure 3) demonstrating that before occlusion, rCBF was relatively heterogeneous and then became more heterogeneous after MCA occlusion.

*NADH Fluorescence*. NADH fluorescence was uniform over the exposed cortex in all groups, measuring 105.3±2.9% before ischemia. After 2 hours of ischemia, NADH fluorescence levels in the ischemic penumbra increased to 152% in both the moderate and severe hyperglycemia groups (P<0.01). In the hypoglycemia group, NADH fluorescence increased to 141±12.8% (P<0.01). After 4 hours of ischemia, NADH fluorescence increased further, to 148±7.1% and 173±16.7% in the moderate and severe hyperglycemia groups, respectively (P<0.01 compared with preischemic values). However, in the hypoglycemia group, NADH redox state improved, measuring 114±7.5%, which was not significantly different from preischemic values. Figure 5 depicts a series of NADH redox state histograms of a hyperglycemic/normoxic animal (Figure 3) demonstrating increased heterogeneity during the period of occlusion.

*Areas of Infarction as Measured With TTC*. Infarct volume in the moderate and severe hyperglycemia groups was 30.1±1.9% and 35.1±3.1% of total hemisphere volume. The hypoglycemia group showed a significantly (P<0.01) smaller infarct volume (13.5±1.9%) than the other 2 groups. Analysis showed that...
hemispheric volumes in all normoxic study groups were not significantly different, indicating that there was no significant early edema formation, which might alter infarct volumes.

Moderately Hypoxic Groups

**Brain pHi.** Brain pHi was uniform over the entire exposed cortex in all groups, measuring $7.00 \pm 0.03$ before ischemia. After 2 hours, brain pHi in the ischemic penumbra fell significantly in the moderate and severe hyperglycemia groups, to $6.50 (P < 0.01$ compared with preischemic values). In the hypoglycemia group, brain pHi declined to $6.87 \pm 0.09$, which was not statistically different from preischemic values. After 4 hours of ischemia, brain pHi declined further, to $6.43 \pm 0.03$ and $6.19 \pm 0.13$ in the moderate and severe hyperglycemia groups, respectively ($P < 0.01$ compared with preischemic values). In the hypoglycemia group, brain pHi measured $6.91 \pm 0.06$, which was not statistically different from preischemic values. Figure 6 depicts a series of histograms of a hypoglycemic/moderately hypoxic animal (Figure 2) demonstrating homogeneity of pHi before occlusion and throughout the ischemic period.

**Regional Cortical Blood Flow.** rCBF in all groups was $48.3 \pm 3.3 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ before ischemia. After 2 hours of ischemia, rCBF fell significantly in the ischemic penumbra ($P < 0.01$) in all groups studied, to $\approx 16 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$. After 4 hours of ischemia, rCBF further declined to $\approx 11 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ in all groups ($P < 0.01$). Figure 6 depicts a series of rCBF histograms of a hypoglycemic/moderately hypoxic animal (Figure 2) demonstrating that before occlusion, rCBF was relatively heterogeneous and then became more heterogeneous with reductions in rCBF after MCA occlusion.

**NADH Fluorescence.** NADH fluorescence was uniform over the exposed cortex in all groups, measuring $101.9 \pm 2.8\%$ before ischemia. After 2 hours of ischemia, NADH fluorescence levels in the ischemic penumbra increased to $\approx 173\%$ in both the moderate and severe hyperglycemia groups ($P < 0.01$ compared with preischemic values). In the hypoglycemia group, NADH fluorescence increased to $111 \pm 9.4\%$. After 4 hours of ischemia, NADH fluorescence increased further, to $167 \pm 10.9\%$ and $197 \pm 22.1\%$ in the moderate and severe hyperglycemia groups, respectively ($P < 0.01$ compared with preischemic values). In the hypoglycemia group, NADH redox state increased slightly, to an overall increase of $130 \pm 13.4\%$, which was not statistically different from preischemic values. Figure 6 depicts a series of NADH redox state histograms of a hypoglycemic/moderately hypoxic animal (Figure 2) demonstrating very little change in the histograms during ischemia.

**Areas of Infarction as Measured With TTC.** Infarct volume in the moderate and severe hyperglycemia groups measured $30.4 \pm 1.2\%$ and $35.0 \pm 1.2\%$ of total hemisphere volume. The hypoglycemia group showed a significantly smaller infarct volume ($21.4 \pm 3.6\%$) compared with the other 2 groups.
Systemic Parameters at 4 Hours of Occlusion

<table>
<thead>
<tr>
<th>Condition</th>
<th>MABP, mm Hg</th>
<th>pH</th>
<th>PaCO₂, mm Hg</th>
<th>PaO₂, mm Hg</th>
<th>ScO₂, %</th>
<th>Lactate, mmol/L</th>
<th>Glucose, mmol/L</th>
<th>HCT, %</th>
<th>Rectal Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>75.8±2.8</td>
<td>7.378±0.016</td>
<td>36.8±1.0</td>
<td>148.2±2.4</td>
<td>99.1±0.1</td>
<td>2.6±0.4</td>
<td>2.4±0.5</td>
<td>34.6±1.5</td>
<td>39.2±0.1</td>
</tr>
<tr>
<td>Moderate hyperglycemia</td>
<td>77.3±1.1</td>
<td>7.369±0.012</td>
<td>38.3±1.0</td>
<td>145.5±8.8</td>
<td>99.0±0.1</td>
<td>3.6±0.7</td>
<td>11.0±0.9</td>
<td>31.0±0.6</td>
<td>38.7±0.4</td>
</tr>
<tr>
<td>Severe hyperglycemia</td>
<td>74.6±5.0</td>
<td>7.308±0.019</td>
<td>40.3±1.1</td>
<td>147.6±8.9</td>
<td>98.6±0.1</td>
<td>9.2±1.3</td>
<td>41.5±5.7</td>
<td>33.4±1.3</td>
<td>38.4±0.4</td>
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<tr>
<td>Moderate hypoxia groups</td>
<td></td>
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<tr>
<td>Hypoglycemia</td>
<td>81.6±5.0</td>
<td>7.372±0.017</td>
<td>38.8±1.6</td>
<td>47.4±1.2</td>
<td>81.3±1.7</td>
<td>3.1±1.0</td>
<td>2.3±0.8</td>
<td>33.0±2.0</td>
<td>39.2±0.1</td>
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<tr>
<td>Moderate hyperglycemia</td>
<td>82.6±1.7</td>
<td>7.377±0.018</td>
<td>38.8±0.6</td>
<td>47.2±2.7</td>
<td>80.6±2.4</td>
<td>8.0±0.7</td>
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<td>34.6±0.9</td>
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<tr>
<td>Severe hyperglycemia</td>
<td>64.8±3.5</td>
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<td>38.7±1.6</td>
<td>51.0±2.3</td>
<td>79.3±3.9</td>
<td>13.3±2.5</td>
<td>52.6±8.8</td>
<td>32.2±1.9</td>
<td>38.4±0.1</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure. Values are mean±SEM; n=5 per group.

(P<0.05). Analysis of hemispheric volumes in all moderate hypoxia study groups were not significantly different, indicating that there was no significant early edema formation, which might alter infarct volumes.

Discussion

The significant findings of the study are as follows. First, in both the normoxia and moderate hypoxia groups, hyperglycemia facilitates development of cortical acidosis in the ischemic penumbra. Second, both moderate and severe hyperglycemia were associated with worsening mitochondrial function in the ischemic penumbra, as assessed by an increase in NADH fluorescence. Alternatively, hypoglycemia led to improvement, with a reduction in NADH redox state. Third, hypoglycemia resulted in a significant reduction in the size of early infarcts as measured by TTC staining. There was not a significant difference in size of infarct between the moderate and severe hyperglycemia groups, because the maximum degree of cortical infarction was realized in both groups. These findings were observed in severe focal cerebral ischemia of 4 hours’ duration without reperfusion. Together, these results support the hypotheses that the development of cortical intracellular acidosis in the ischemic penumbra is dependent on glucose utilization and that this acidosis leads to increases in cortical infarction size.

Hyperglycemia

Hyperglycemia has been known to worsen neurological damage in different models of cerebral ischemia. In clinical studies, diabetic patients who have experienced a stroke appear to have a significantly worse outcome than nondiabetic patients.\(^{23-25}\) Mortality rates have been shown to be increased in acute stroke patients who have fasting levels of glucose >6.1 mmol/L.\(^{26}\) In a more recent study, Toni et al\(^{27}\) suggested that when serum glucose levels were lowered in diabetic stroke patients, a better outcome was achieved.

A number of investigators have studied the effects of hyperglycemia in models of focal cerebral ischemia. These studies have shown conflicting results, either worsening of ischemic damage\(^{11,15,28-32}\) or amelioration of ischemic damage.\(^{13,33-37}\) The discrepancy in these studies could be explained in part by the degree of collateral blood supply. In models of focal “incomplete” cerebral ischemia, in which there was some collateral flow (“trickle flow” phenomenon), hyperglycemia appeared to exacerbate ischemic damage.\(^{15,31,38}\) However, in other studies of focal cerebral ischemia, in which there was a greater amount of collateral blood flow, the effects of hyperglycemia on ischemic damage were less.\(^{15,34,35}\) In our study of the ischemic penumbra, increasing the serum glucose levels before the onset of focal ischemia resulted in greater brain intracellular acidosis, increased the heterogeneity of pH\(_i\), adversely affected NADH redox state, and significantly increased infarct volume compared with the hypoglycemia group. The increased heterogeneity of pH\(_i\) with increasing plasma glucose concentration observed in this study is in agreement with that of LaManna et al.\(^{2}\) In a model of cardiac arrest and using neutral red histophotometry, they showed that when the plasma glucose concentration was increased, there was greater acidosis and increased heterogeneity because of greater accumulation of tissue lactate. They also demonstrated that during ischemia, pH\(_i\) and tissue lactate accumulation are linearly correlated. Griffith et al\(^{1}\) also showed increased heterogeneity during ischemia in a model of global ischemia.

Hypoglycemia

Preischemic moderate hypoglycemia in models of focal cerebral ischemia has not been studied as extensively as in models of global and forebrain ischemia. It is known that hypoglycemic coma without occlusion of the MCA results in neuronal damage in selective areas of the brain.\(^{39-41}\) Kristián et al\(^{41}\) showed that during hypoglycemic coma, brain pH\(_i\) did not become acidic when the animals were normocapnic, although there were selective areas of neuronal damage. When the animals were made hypercapnic, however, brain pH\(_i\) became more acidic, with greater regions of neuronal damage and pan-necrosis. In a model of forebrain ischemia, Smith et al\(^{42}\) showed that during ischemia, brain pH\(_i\) was less acidic in animals with hypoglycemia than in animals with normoglycemia, 6.37±0.04 versus 6.15±0.06. In this study, the plasma glucose level during hypoglycemia was 4.6±0.1 mmol/L. Conversely, in a model of global ischemia using 4-vessel occlusion, Nagai et al\(^{42}\) noted that pH\(_i\), which became acidic during ischemia, was not different in either the normoglycemic or hypoglycemic setting. The plasma...
glucose level was not specified in that study. There have been several reports in which insulin reduced ischemic damage.43–45 Hamilton et al 45 showed that by reducing the blood glucose to minimum values but within the physiological range (moderate hypoglycemia), infarct volume could be significantly reduced. In this study, untreated animals had a cortical infarct volume of 39.9±7.3 mm³, whereas the insulin-treated animals had reductions in infarct volume to 22.5±3.1 mm³ (43.5%). This is in close agreement with our study, in which we showed a reduction in infarct volume by 47.7%. Furthermore, this experiment demonstrates that hypoglycemia or the avoidance of hyperglycemia may be beneficial for neurosurgical procedures in preventing ischemic damage as a result of temporary arterial occlusion.46

Moderate Hypoxia/Ischemia

Many studies have investigated the effects of hypoxia on the brain.46–53 Some of these studies were done in animals made hypoglycemic or hyperglycemic.50,51 Other studies used the Levine rat preparation, which is a model of global ischemia with hypoxia as a method to further exacerbate tissue damage.45 To the best of our knowledge, no studies of moderate hypoxia in focal cerebral ischemia have been performed. In our study, we noted 3 distinct findings in animals studied with moderate hypoxia: (1) there was no difference in infarct volume, pHᵢ, or NADH redox state between the normoxic and moderately hypoxic animals during moderate hyperglycemia; (2) moderate hypoxia exacerbated brain intracellular acidosis compared with the normoxic animals in the severe hyperglycemia group; and (3) in hypoglycemia groups, although brain pHᵢ was slightly but not significantly alkalotic in the moderate hypoxia group, infarct volume was 180% greater than in the normoxia group. It has been documented that during moderate hypoxia (Pao₂ ≈45 to 55 mm Hg), there are biochemical alterations in brain tissue levels of energy metabolites, NADH redox state, and pHᵢ, ATP, ADP, and AMP do not change unless the Pao₂ is <25 mm Hg, whereas phosphocreatine, NADH redox state, and pHᵢ change at <35 mm Hg.52,55 However, the lactate and pyruvate levels begin

![Image of graphs showing brain pHᵢ, NADH redox state, cerebral blood flow, and cortical infarct volume under various glucose and hyperglycemia conditions. The graphs illustrate the effects of hypoglycemia and hyperglycemia on cerebral ischemia and the significance of pHᵢ and NADH changes in brain tissue.](http://stroke.ahajournals.org/doi/pdf/10.1161/01.STR.90.1.166)
to increase when the PaO₂ is <50 mm Hg. This would explain in part why the severely hyperglycemic/moderately hypoxic animals were markedly more acidic than the severely hyperglycemic/normoxic animals. It is interesting to note that the increase in NADH fluorescence between the hypoglycemia and hyperglycemia groups was not enhanced with the addition of moderate hypoxia. The results of this study suggest that one adverse effect of acidosis is increased damage to mitochondria. It has been shown previously that during complete and incomplete ischemia, lactic acidosis prevents normalization of mitochondrial respiration. Wagner et al demonstrated that a combination of transient anoxia in hyperglycemic cats caused altered mitochondrial respiration. Our study would support the above findings and conclusion that acidosis does adversely alter mitochondrial function during the acute ischemic insult. In fact, the observation that changes in systemic O₂ did not significantly alter NADH fluorescence supports the contention that the observed effects were not due to a failure of substrate delivery for aerobic metabolism but rather a direct effect of hyperglycemia on the mitochondria. The mechanisms by which acidosis might adversely affect mitochondria have been expertly discussed elsewhere.
The effects of hyperglycemia on NADH redox state occurred acutely in our experiment, suggesting that free radical formation was not the cause but rather that other mechanisms, such as mitochondrial calcium overload, were at play.

**TTC Staining Technique**

Tetrazolium salts have been used to determine the area and degree of infarction in myocardial tissue obtained from patients. This technique has been extended for use in experimental animals in the study of cerebral injury as a result of unilateral temporary or permanent MCA occlusion. TTC is a water-soluble salt that is reduced to formazan by the enzyme succinate dehydrogenase in mitochondrial tissue. This in turn stains a deep red color in normal tissue. In ischemic tissue in which mitochondria have been damaged, however, there would be a lack of staining, i.e., tissue will be a white or pale color.

The reliability of TTC staining as an early marker (<24 hours after MCA occlusion) of ischemic damage has been controversial.

**Figure 6.** Composite histogram of experiment in Figure 2. Before ischemia, the distribution of rCBF is quite heterogeneous compared with brain pH, or NADH redox state. Over the next 2 hours after MCA occlusion, rCBF becomes less heterogeneous, then at hours 3 and 4 becomes more heterogeneous, but with rCBF values significantly less than preischemic values. The distribution of pH, is unchanged during the 4-hour period of occlusion. The distribution of NADH redox state became less homogeneous over the 4-hour period of occlusion.
Infarction can be detected as early as within 1 to 2 hours; however, the color differences between red and white are subtle, making it difficult to delineate the extent of infarction. At infarct times of ≥3 hours, the infarcted tissue becomes distinctly delineated even before development of histological evidence of infarction. Hatfield et al. showed that 5 to 20 minutes after MCA occlusion, the area of damage as assessed by hematoxylin-eosin staining was significantly smaller than that assessed by TTC. At 3 to 4 hours after MCA occlusion, however, there was good correlation between hematoxylin-eosin and TTC staining. At 24 hours, infarct size was not significantly different from the 3 to 4 hours post–MCA occlusion group. It can be postulated that when the animals are killed 5 to 20 minutes after MCA occlusion, the cerebral metabolic rate of oxygen is reduced immediately after occlusion. TTC can overestimate infarct size because, although mitochondrial function is compromised, it may potentially recover. Peri-infarct edema can also affect assessment of infarct volume. Other studies have also shown similar results.

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References
Intracerehmic hyperglycemia has been widely demonstrated to exacerbate brain injury in a variety of animal models by enhancement of intracellular acidosis, consequent loss of ion homeostasis, and through bioenergetic failure. A host of potential cellular mechanisms have been identified, with particularly good evidence for the involvement of iron-catalyzed production of reactive oxygen species. It is increasingly clear that lactic acidosis amplifies multiple subcellular, and potentially mitochondrial, injury cascades during cerebral ischemia. However, results in focal cerebral ischemia have not completely agreed on the deleterious effect of high glucose, in part because glycemic hyperglycemia increases infarct size in collaterally perfused but not end-arterial vascular territories.


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