Intracellular Acidosis During and After Cerebral Ischemia: In Vivo Nuclear Magnetic Resonance Study of Hyperglycemia in Cats

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In vivo $^{31}$P nuclear magnetic resonance spectroscopy was used to monitor the time course of intracellular pH in cat cerebral cortex subjected to global cerebral ischemia under control and hyperglycemic pretreatment conditions. Transient (16 minutes) global cerebral ischemia was induced in 14 cats using an inflatable cervical cuff combined with systemic arterial hypotension. Six cats were pretreated with infusion of 1.5 g/kg glucose prior to ischemia. Relative concentrations of high-energy phosphate metabolites and intracellular pH were continuously monitored before, during, and for 2 hours after cerebral reperfusion. During ischemia, intracellular pH fell to the same level and followed a similar time course in both groups. However, during initial reperfusion in the hyperglycemic group, there was a severe further decline ($p<0.003$) in intracellular pH. We suggest that the increased neurologic deficit and mortality found in hyperglycemic animals subjected to cerebral ischemia may be attributed to this transient severe tissue acidosis. (Stroke 1987;18:919-923)

Intracellular acidosis is considered to be a critical factor in ischemic cell damage.1-3 Metabolic studies in a cat model of transient global ischemia have suggested that high glucose levels accentuate cell damage by further enhancing anaerobic glycolysis and lactic acidosis. The resulting severe intracellular acidosis is incompatible with cell survival.4-6 In the present study, $^{31}$P nuclear magnetic resonance (NMR) spectroscopy permitted dynamic monitoring of intracellular pH and the corresponding changes in high-energy metabolites not possible in previous studies. Using a cat model of transient global ischemia, we suggest that the difference in pH between control and hyperglycemic cats at a critical time early after reperfusion may explain the deleterious effect of glucose.

**Materials and Methods**

Fourteen female cats (2.4-3.4 kg) were used, 8 for control and 6 for hyperglycemia studies. Cats were fed only water ad libitum overnight. Anesthesia was induced with 4% halothane and was followed by a tracheotomy and i.v. injection of 0.02 mg atropine and 0.08 mg/kg pancuronium bromide. The cats were mechanically ventilated using 0.08% halothane/33% O$_2$/66% N$_2$O + CO$_2$. Ventilation gases were adjusted to maintain blood gases within the physiologic range. Rectal temperature was monitored and maintained at 37° C using a heating blanket. Electroencephalograms (EEGs) were recorded continuously from both hemispheres. Both femoral arteries and veins were cannulated for monitoring arterial pressure and blood gases, and/or for sampling glucose and lactate, and for infusion of drugs.

A cervical cuff was used to induce complete global cerebral ischemia.7 A tourniquet, similar to a miniature pressure cuff, was wrapped around the cat's neck and inflated to 35 psi to induce 16 minutes of global cerebral ischemia. Trimethaphan camsylate (Arfonad, Roche Laboratories, Nutley, N.J.) was used to induce rapid systemic arterial hypotension (<50 mm Hg) immediately before cuff inflation and to control blood pressure during ischemia. Positive end-expiratory pressure was used to maintain systemic arterial hypotension during cuff inflation. Immediately before cuff deflation, the blood pressure was elevated and maintained by infusion of norepinephrine (Levophed, Winthrop-Breon, New York, N.Y.).

A 1.89-T superconducting 60-cm bore magnet with a Bruker Biospec console (Billerica, Mass.) was used for the $^{31}$P NMR studies. Before cuff inflation, the cats were placed in the nonferromagnetic holder and placed in the magnet for NMR spectroscopy. A 2-cm, 2-turn double-tuned surface coil (proton and phosphorus resonance) was placed within 2 mm over the parietal cortex. Thus, the sensitive volume of tissue providing the signal extended to a subcortical depth of approximately 8 mm. To obtain $^{31}$P spectra, a 40-μsec, 90° pulse was applied to the coil. An interpulse delay of 1.55 seconds was used. One hundred twenty-eight transients were averaged over 4 minutes. The spectral width was 4,000 Hz, and the data length was 4 k. Transients were processed with a 15-Hz Gaussian line broadening factor and a 200-Hz exponential multiplication factor. Partial saturation of spectral intensities, particularly those with long T1 [e.g., phosphocreatine

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(PCr)), resulted from the short recycling time. Four 4-
minute scans were obtained for control spectra. Glu-
cose infusion was followed by 4 additional 4-minute
scans. Spectra were continuously obtained during
ischemia and continued for 1.5-4 hours after reper-
fusion.

Four preischemia (control), 4 preischemia-postinfu-
sion, and 4 90 ± 8 minutes postischemia spectra were
averaged to improve the signal-to-noise ratio to permit
determination of spectral peak intensities. Spectral pa-
rameters calculated were ratios of phosphocreatine to
inorganic phosphate (PCr:Pi), PCr:β-ATP, and total
adenylate intensity. The PCr:Pi and PCr:β-ATP ratios
are useful indexes for the status of energy metabolism.8
Intracellular pH was determined by the chemical shift
of Pi from PCr.9,10

For the hyperglycemia study, 50% dextrose was
infused i.v. at 0.2 ml/min to a total of 1.5 g/kg before
ischemia. Arterial and venous blood were taken for
measurement of glucose and lactate concentrations at
control times.

All measurements are presented as means ± SD.
The level for rejection of the hypothesis for paired t
tests was 0.05/no. of tests. Student's t
tests were used
for intergroup comparisons, with
p<0.05 needed for
significance. With unequal variances, Welch's
t test
was used.

Results

Blood gas and mean blood pressure values for both
groups are presented in Table 1. Preinfusion and con-
trol serum glucose levels were 160 ± 74 mg/dl. In cats
administered glucose, preischemia serum levels rose
to 457 ± 85 mg/dl (p<0.001).

EEG tracings became isoelectric ≤15 seconds after
the induction of ischemia. Arterial hypotension in-
duced during ischemia was successfully reversed in
both groups. During postischemia reperfusion, recov-
ery of EEG activity was variable. Of the 7 cats in
which EEG was monitored, 1 exhibited isoelectric
EEG at 2 hours postischemia. EEG activity returned in
the remaining 6 cats, but tended to remain abnormal,
exhibiting reduced amplitude, slowed activity, and, in
3 cats, periodic bilateral bursts of activity. Given the
small sample size, no effort was made to correlate the
EEG data with other data.

Figure 1 shows representative 31P spectra at times
during the experimental sequence. The control spec-
trum exhibits resonant peak intensities attributable to
phosphomonoesters, Pi, phosphodiester, PCr, and γ-,
α-, and β-ATP. The spectrum at 12 minutes of ische-
mia reveals an increase in the Pi intensity and no
NMR-detectable ATP. Within 4 minutes of cerebral
reperfusion, PCr and ATP intensities became observ-
able by NMR, increased in magnitude, and returned to
control values within 1 hour postischemia. The time
courses of adenylate, PCr, and Pi NMR intensity
changes for both hyperglycemic and control groups
were nearly identical at all times in ischemia and re-
cover. Table 2 presents the values of PCr:Pi, PCr:β-
ATP, and the sum of the adenylate peak intensities
measured as a fraction of the pretreatment baseline
values for the control and hyperglycemic groups.
These values were averaged over the preischemic post-
infusion intervals and during 90 ± 8 minutes of reper-
fusion. No significant differences in the normalized

Table 1. Blood Gases and Mean Arterial Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>Arterial blood pressure (mm Hg)</th>
<th>pH</th>
<th>Pco2 (mm Hg)</th>
<th>Po2 (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hyper-glycemia</td>
<td>Control</td>
<td>Hyper-glycemia</td>
</tr>
<tr>
<td>Preischemia</td>
<td>112 ± 12</td>
<td>105 ± 7</td>
<td>7.33 ±0.07</td>
<td>7.29 ±0.07</td>
</tr>
<tr>
<td>Preischemia-postinfusion</td>
<td>—</td>
<td>99 ± 11</td>
<td>—</td>
<td>7.33 ±0.07</td>
</tr>
<tr>
<td>Recirculation (16 min)</td>
<td>99 ± 6</td>
<td>99 ± 10</td>
<td>7.22 ±0.07*</td>
<td>7.26 ±0.07</td>
</tr>
<tr>
<td>Recirculation (90 min)</td>
<td>101 ± 19</td>
<td>100 ± 13</td>
<td>7.27 ±0.19</td>
<td>7.28 ±0.10</td>
</tr>
</tbody>
</table>

*p<0.01 compared with preischemia.
PCr:\Pi and PCR:β-ATP ratios were found between preischemic steady state and 90 minutes of reperfusion, both within and between the hyperglycemic and control groups. However, the normalized sum of the adenylate peak intensities after recirculation in the control and hyperglycemic groups declined 20% (p<0.007) and 13% (p<0.01), respectively, from preischemic baseline values.

Dynamic changes in pH for both experimental groups before, during, and after ischemia are illustrated in Figure 2. Infusion of glucose in the hyperglycemic group did not affect preischemic pH compared with preinfusion values; there were no significant differences in pH between groups at any times prior to ischemia. During ischemia, pH fell to an equal degree at similar times in both groups. However, during the initial times following reperfusion, there was a significant further decline in pH from the minimum pH observed during ischemia (p<0.003) that was seen only in the hyperglycemic cats; this decline was significantly greater than that observed in the control group (p<0.015). There was no correlation of the maximum further pH decrease in individual cats with preischemic hyperglycemia. Although showing no further decline in pH, the control group did exhibit a delay in pH recovery during reperfusion that was similar to that in hyperglycemic cats. The lowest pH observed during reperfusion occurred at 9.0 ± 2.1 and 8.6 ± 1.5 minutes for control and hyperglycemic cats, respectively (not significant). The mean time to recovery of 99% of control pH was also nearly identical, 48 ± 5.8 and 48.6 ± 8.2 minutes for the control and hyperglycemic groups, respectively (not significant).

**Table 2.** PCr:Pi, PCR:β-ATP, and Sum of Adenylate Intensities as Fraction of Pretreatment Baseline Values at Preischemia-Postinfusion and Reperfusion for Control and Hyperglycemic Cats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr:Pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia-postinfusion</td>
<td>—</td>
<td>0.95 ± 0.17</td>
</tr>
<tr>
<td>Recirculation (90 ± 8 min)</td>
<td>0.71 ± 0.44</td>
<td>1.01 ± 0.23</td>
</tr>
<tr>
<td>PCr:β-ATP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia-postinfusion</td>
<td>—</td>
<td>1.09 ± 0.24</td>
</tr>
<tr>
<td>Recirculation (90 ± 8 min)</td>
<td>1.04 ± 0.28</td>
<td>1.16 ± 0.41</td>
</tr>
<tr>
<td>Total adenylates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia-postinfusion</td>
<td>—</td>
<td>0.80 ± 0.13*</td>
</tr>
<tr>
<td>Recirculation (90 ± 8 min)</td>
<td>0.94 ± 0.07</td>
<td>0.87 ± 0.08††</td>
</tr>
</tbody>
</table>

**Discussion**

Pretreatment of experimental animals with glucose prior to complete ischemia accentuates postischemic brain dysfunction, possibly caused by a worsening of cellular acidosis, although the precise mechanisms are unclear. Our data have shown that this cannot be explained by brain pH changes before or during ischemia, or later, after reperfusion. Nor can it be explained by differential effects on high-energy phosphate metabolism. The major difference between glucose-pretreated and control cats was a significant further decline in intracellular pH early after the onset of reperfusion of the previously ischemic brain. Although we obtained neither histologic nor behavioral measures in the present experiments, we postulate that the increase in morbidity of glucose-treated animals reported in other studies may be attributed to this critical lowering of pH.

Our studies do not directly address the biochemical basis of the brain acidosis or the mechanisms by which this may cause cell damage. However, anaerobic glucose metabolism and consequent lactic acidosis are well known. Lactic acidosis generates excess hydrogen ions and thereby results in a severe lowering of intracellular pH if cellular buffering capacities are exceeded and/or compromised. In the present study, prolonged acidosis seen after transient complete ischemia could indicate a delay in the brain's ability to switch from anaerobic to aerobic metabolism. The restored delivery of glucose during reperfusion of brain that is still metabolizing anaerobically encourages a persistent state of brain lactic acidosis. Although plasma glucose at 15 minutes of reperfusion is only approximately 20% higher in glucose-pretreated cats, our studies imply that this provides the extra margin of glucose to worsen lactic acidosis.

In cats subjected to 15 minutes of global ischemia with measurements made after up to 90 minutes of reperfusion, cortical lactate levels were approximately 40 mmol/kg or less in untreated cats. However, in the present study, which used a similar model and similar time periods, no difference in pH was observed between control and hyperglycemic cats at 90 minutes of reperfusion. Furthermore, brain pH had returned to control values, indicating recovery of cellular buffering capacity. Taking the two studies together, if the further decline in pH on reperfusion in hyperglycemic cats is caused by greater lactate accumulation, then the lactate concentration may exceed 40 mmol/kg in the
early period after reperfusion. Even if this were not so, the differential accumulation of lactate is likely to commence much earlier than 90 minutes after reperfusion, when brain buffering capacity is impaired. Proton NMR spectroscopy of brain lactate is currently being performed to clarify this issue.

Prolonged cerebral acidosis after transient ischemia has been reported previously by Anderson and Sundt in a monkey model of focal incomplete cerebral ischemia. Using fluorescent indicators in halothane-anesthetized monkeys, they demonstrated a fall in intracellular pH during ischemia and reperfusion. However, a smaller decline in pH during ischemia and no increased postischemic acidosis were found in monkeys anesthetized with barbiturate rather than halothane. Indirectly, this would support excessive lactate accumulation being responsible for tissue acidosis since barbiturate anesthesia decreases the rate of tissue lactate accumulation. That study also involved the continuous delivery of glucose to the brain despite arterial occlusion due to incomplete ischemia, which causes a gradual accumulation of brain lactate and increased tissue acidosis over time. Thus, there may be some similarity in the mechanisms of neurologic damage caused by hyperglycemia during complete ischemia compared with that caused by normoglycemia during incomplete ischemia.

By what mechanism the further decline in intracellular pH recorded in hyperglycemic animals can explain the greater cellular damage can only be speculative. Extracellular pH microelectrode measurements after tourniquet-induced global cerebral ischemia and reperfusion in rats also recorded prolonged acidosis (pH 6.1–6.2) and, in some rats, a further transient fall in brain pH after reperfusion. Electrode measurements of extracellular pH in rats pretreated with glucose showed that although pH fell to similar values during ischemia, a secondary increase in hydrogen ion concentration occurred during reperfusion, when pH values fell as low as 5.4. When combined with our own intracellular pH measurements, these data suggest that, in hyperglycemia, the cellular hydrogen ion buffering capacity undergoes severe and abrupt deterioration, allowing pH to fall to levels that could be critical for the activation of cell lysosomes, and thus may explain how glucose exaggerates ischemic cell damage.

Cerebral pH is obtained in NMR spectroscopy by measuring the chemical shift difference between Pi and PCr. An area of concern is the effect of ion concentration on the Pi titration curve; the concentration of Mg can affect the chemical shift of Pi. Petroff et al. have studied the effect of different concentrations of Mg on Pi solutions. They found that the titration curve was affected only at concentrations >5.8 mM Mg, well above the levels of 0.5–1.5 mM Mg thought to be present in the brain cytosol. During ischemia and initial reperfusion, concentrations of free Mg may increase as ATP levels fall and can conceivably alter pH values measured by NMR. However, intracellular pH values measured by NMR during extreme acidosis are consistent with the extracellular pH values reported by Kraig et al. This finding suggests that the activity of free Mg during and after cerebral ischemia does not significantly affect pH values obtained by NMR. Thus, the chemical shift of P Cr is probably not significantly affected at the acidic pH values achieved in the present experiment.

Our data also indicate a decline of total adenylate concentration at 90 minutes of reperfusion from pre-ischemic levels in both the control and hyperglycemic cats. This decline is consistent with other investigations, in which an overall depression of 20% in ATP has been reported. It is possible that this depression represents a retained coupling between glucose metabolism and functional activity in the brain at 90 ± 8 minutes of reperfusion, and that the decrease in ATP concentration merely reflects an altered state of excitability. This is suggested by the return of pH and PCr:Pi and PCr:β-ATP ratios to control levels, which in turn reflects the return to phosphorylation equilibrium conditions.

In summary, in vivo 31P NMR spectroscopy of ischemic cat brain pretreated with glucose showed that, on reperfusion, there was a significant, although transient, further increase in intracellular acidosis that was not found in control cats. We suggest that the increased neurologic deficit and increased mortality found in hyperglycemic animals subjected to ischemia may be attributed to this transient severe tissue acidosis occurring at a critical time early after reperfusion. We further speculate that cell damage may be expedited by enhanced lactic acidosis, which exceeds intracellular hydrogen ion buffering capacity and depresses intracellular pH levels below those critical for lysosome activation.

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References


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