Global Cerebral Ischemia and Intracellular pH During Hyperglycemia and Hypoglycemia in Cats

M. Chopp, PhD, K.M.A. Welch, MD, C.D. Tidwell, BS, and J.A. Helpern, PhD

In 27 cats treated to vary arterial serum glucose concentrations, we measured cerebral high-energy phosphate metabolite concentration and intracellular pH using in vivo phosphorus-31 nuclear magnetic resonance spectroscopy during transient global cerebral ischemia and reperfusion. Hypoglycemia was induced with 4 units/kg i.v. insulin in six cats before ischemia; hyperglycemia was induced with 1.5 g/kg i.v. glucose in six cats before and in six cats during ischemia. Nine untreated cats subjected to ischemia without manipulation of blood glucose concentration served as controls. During ischemia, intracellular pH fell to similar levels in the control and both hyperglycemic groups. During reperfusion, the hyperglycemic before ischemia group initially exhibited a severe further decline in intracellular pH (p<0.003); this further decline was not observed in the control or the hyperglycemic during ischemia groups. Intracellular acidosis was attenuated both during ischemia and early after reperfusion in the hypoglycemic before ischemia group. In all groups, cerebral high-energy phosphate metabolite concentrations were depleted during ischemia and then recovered to the same degree during reperfusion. Our data suggest that brain glucose stores before ischemia determine the severity and time course of intracellular acidosis during ischemia and reperfusion. (Stroke 1988;19:1383-1387)

The influence of systemic blood glucose concentration on the neurologic outcome of stroke has been repeatedly documented in experimental animals and patients.1-8 Experiments have suggested that this effect is mediated by brain acidosis.8-12 Efforts to control blood glucose concentration could have promise in the clinical management of stroke, but the precise conditions under which this might be accomplished remain uncertain. For example, if preexisting high blood and tissue glucose concentrations dictate an unfavorable outcome, control of hyperglycemia after stroke onset will be ineffective. However, if control of blood glucose concentration is worthwhile, should levels be controlled to the normal range and will subnormal (even hypoglycemic) levels produce added benefit? We have begun to address these questions by observing energy metabolism and pH in an animal model of global ischemia using noninvasive in vivo phosphorus-31 nuclear magnetic resonance spectroscopy (31P NMR), a technique that has potential for the dynamic monitoring of stroke patients who might be candidates for manipulation of systemic glucose concentrations.

Materials and Methods

We studied 27 female cats weighing 2.2–3.4 kg. The cats were fasted for 24 hours, with water given ad libitum. Anesthesia induced with 4% halothane in O2 was followed by tracheotomy, intravenous injection of 0.02 mg atropine and 0.08 mg/kg pancuronium bromide, and mechanical ventilation with <1% halothane/33% O2/66% N2O+CO2. Ventilation gases were adjusted to maintain blood gases within the physiologic range. Rectal temperature was monitored and maintained at 38°C. Both femoral arteries and veins were cannulated to monitor arterial blood pressure and blood gases, to sample blood for arterial glucose concentration, and to intravenously infuse drugs as needed. A tourniquet, constructed similar to a miniature blood pressure cuff, was wrapped around the cat’s neck and inflated to 35 psi to induce 16 minutes of transient global cerebral ischemia.13 Trimethaphan camsylate (Arfonad, Roche Laboratories, Nutley, New Jersey), 4 mg/ml i.v., was given and titrated to induce rapid systemic arterial hypotension (40–50 mm Hg) immediately before cuff inflation; positive end-expiratory
pressure maintained systemic arterial hypotension (<50 mm Hg) during cuff inflation. Immediately before cuff deflation, the blood pressure was elevated and maintained, when necessary, during recirculation by infusion of 0.1 mg/ml norepinephrine (Levophed, Winthrop-Breon Laboratories, New York, New York). The total amount of norepinephrine administered over the 2 hours of recirculation varied between 0 and 7 ml for all cats, with the majority receiving approximately 3 ml.

Prior to placement of the cat in the magnet, the skull over the parietal cortex was exposed. All muscle within 3 cm of the surface coil was cleared to eliminate contamination of the NMR spectra. A Bruker Biospec console (Karlsruhe, FRG) was used to eliminate contamination of the NMR spectra. A 2-cm, two-turn, double-tuned surface coil (proton and phosphorus resonance) was placed over the parietal cortex with the exposed skull intact. The NMR spectra were obtained using a 40-μsec effective 90° pulse applied to the coil; 128 transients over 4 minutes were averaged. Spectral width was 4,000 Hz. Before manipulation of blood glucose concentration by infusion of glucose/insulin, four baseline spectra were obtained; after infusion but before induction of ischemia four more spectra (postinfusion-preischemia) were obtained. Spectra were continuously obtained during ischemia and for 2.0 hours of recirculation. Spectra were processed with a profile-correction routine14 supplied by Bruker and 20-Hz exponential line broadening.

Cats were divided into four groups: 1) control, nine untreated cats; 2) hyperglycemic before, six cats that received 1.5 g/kg i.v. D-50 glucose 1–1.5 hours before ischemia; 3) hyperglycemic during, six cats that received 1.5 g/kg i.v. D-50 glucose during ischemia; and 4) hypoglycemic, six cats that received 4 units/kg i.v. porcine insulin 1.5–2.0 hours before ischemia. Data from seven of the nine control and the six preischemia hyperglycemic cats have been reported.15

The four baseline spectra, the four postinfusion-preischemia spectra, and four 90±8 minutes recirculation spectra were averaged to improve the signal-to-noise ratio and permit determination of spectral peak heights. Every 4 minutes during ischemia and recirculation, intracellular pH was calculated from the chemical shift of inorganic phosphate (P_i) from phosphocreatine (PCr).16,17 The use of in vivo 31P NMR to assess brain pH has been confirmed by a number of investigators.18,19 The absolute accuracy of this technique is estimated to be ±0.1 pH unit, whereas changes in pH can be measured to ±0.05 pH unit.20 At a pH of <6.0, the standard curve for relating the chemical shift of P_i from PCr begins to flatten.17,18 We estimate that this reduces the precision of measuring pH differences to approximately ±0.15 pH unit.

All data are presented as mean ± SD. The probability level for rejecting the null hypothesis for paired t tests was 0.05/number of tests. Student's t tests were used to compare groups, with p<0.05 needed for significance. With unequal variances, Welch's t test was used.21

Profile analysis was performed on intracellular pH data from the four ischemic times and first three recirculation times as well as on arterial glucose concentration data. If profile analysis indicated significant interaction effects, subanalyses were performed using Bonferroni's multiple comparison adjustment for each subanalysis.21

Results

Table 1 presents physiologic data for each group. The control group exhibited a significant decline from baseline in arterial pH after 16 minutes of recirculation. Profile analysis of arterial glucose concentration revealed a significant interaction (p<0.001) among all groups at all times noted in Table 1. Analysis of variance (without repeated measures) comparing the groups within times in Table 1 revealed no significant differences among groups at baseline. Comparison of the two hyperglycemic groups revealed a significant difference (p<0.013) in glucose concentration after 16 minutes of recirculation but no significant difference after 90 minutes of recirculation. Significant differences (p<0.001) in arterial glucose concentration was found between all other groups at 16 and 90 minutes of recirculation.

Figure 1 shows representative 31P spectra at baseline, during ischemia, and twice during recirculation. The spectra exhibited seven resonance peaks attributed to phosphomonoesters, P_i, phosphodiesters (sugar phosphates), PCr, and adenylates composed mostly of γ, α, and β-ATP. During ischemia, there was a rapid increase in the P_i and a rapid (within 4 minutes), marked decline in the PCr and the three adenylate peaks. On recirculation, the three adenylate peaks were again demonstrated within 4 minutes and returned to baseline values in all groups within 40 minutes. No significant differences in the peak heights of P_i, PCr, β-ATP, PCr/β-ATP, and PCr/P_i averaged over 90±8 minutes of recirculation as a percentage of baseline were found between groups. Likewise, no significant differences in these spectral peak heights as a percentage of baseline were found between the hyperglycemia before and the hypoglycemia groups. Although a recent report22 suggests that circulating catecholamines modulate ischemic brain damage, we observed no correlation between amount of norepinephrine infused and metabolic outcome.

Figure 2 illustrates the intracellular pH for the groups at baseline, during ischemia, and during recirculation. Figure 2, top, shows pH for the control, hyperglycemic before, and hypoglycemic groups; Figure 2, bottom, compares the hyperglycemic before and the hyperglycemic during groups. There were no
TABLE 1. Arterial Blood pH, Gases, Pressure, and Serum Glucose Concentrations in 27 Cats Subjected to 16-Minute Global Cerebral Ischemia

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>pH</th>
<th>( \text{PaCO}_2 ) (mm Hg)</th>
<th>( \text{PaO}_2 ) (mm Hg)</th>
<th>Pressure (mm Hg)</th>
<th>Glucose concentration (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.33 ± 0.06</td>
<td>31.9 ± 2.6</td>
<td>149 ± 24</td>
<td>114 ± 10</td>
<td>141 ± 40</td>
</tr>
<tr>
<td>16 min</td>
<td>7.20 ± 0.08*</td>
<td>44.2 ± 14.5</td>
<td>129 ± 35</td>
<td>106 ± 17</td>
<td>129 ± 32</td>
</tr>
<tr>
<td>90 min</td>
<td>7.25 ± 0.16</td>
<td>31.4 ± 9.5</td>
<td>135 ± 29</td>
<td>106 ± 21</td>
<td>166 ± 82</td>
</tr>
<tr>
<td><strong>Hyperglycemic before (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.29 ± 0.07</td>
<td>30.2 ± 8.2</td>
<td>166 ± 12</td>
<td>105 ± 7</td>
<td>177 ± 59</td>
</tr>
<tr>
<td>Postinfusion-preischemia</td>
<td>7.33 ± 0.07</td>
<td>29.1 ± 8.0</td>
<td>168 ± 13</td>
<td>99 ± 11</td>
<td>457 ± 85†</td>
</tr>
<tr>
<td>Recirculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 min</td>
<td>7.26 ± 0.07</td>
<td>30.0 ± 4.0</td>
<td>133 ± 40</td>
<td>99 ± 10</td>
<td>499 ± 86‡</td>
</tr>
<tr>
<td>90 min</td>
<td>7.28 ± 0.10</td>
<td>30.9 ± 8.4</td>
<td>152 ± 39</td>
<td>100 ± 13</td>
<td>398 ± 66‡</td>
</tr>
<tr>
<td><strong>Hypoglycemic (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.31 ± 0.07</td>
<td>35.2 ± 9.3</td>
<td>158 ± 8</td>
<td>103 ± 5</td>
<td>108 ± 17</td>
</tr>
<tr>
<td>Postinfusion-preischemia</td>
<td>7.26 ± 0.06</td>
<td>34.0 ± 3.4</td>
<td>173 ± 26</td>
<td>98 ± 4</td>
<td>30.3 ± 9§</td>
</tr>
<tr>
<td>Recirculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 min</td>
<td>7.29 ± 0.06</td>
<td>33.8 ± 7.0</td>
<td>152 ± 71</td>
<td>107 ± 10</td>
<td>72 ± 28</td>
</tr>
<tr>
<td>90 min</td>
<td>7.29 ± 0.09</td>
<td>32.9 ± 4.1</td>
<td>158 ± 13</td>
<td>106 ± 9</td>
<td>78 ± 48</td>
</tr>
<tr>
<td><strong>Hyperglycemic during (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.36 ± 0.08</td>
<td>31.8 ± 3.5</td>
<td>134 ± 21</td>
<td>110 ± 9</td>
<td>197 ± 23</td>
</tr>
<tr>
<td>Recirculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 min</td>
<td>7.18 ± 0.07</td>
<td></td>
<td>36.5 ± 6.8</td>
<td>148 ± 32</td>
<td>117 ± 31</td>
</tr>
<tr>
<td>90 min</td>
<td>7.30 ± 0.07</td>
<td>31.7 ± 4.1</td>
<td>120 ± 21</td>
<td>108 ± 9</td>
<td>526 ± 188 ‡</td>
</tr>
</tbody>
</table>

* † ‡ § || p < 0.002, 0.0007, 0.0001, 0.0006, 0.01, 0.0003, respectively, different from baseline by paired t test.

Discussion

In summary, our results indicate that hyperglycemia before the induction of global cerebral ischemia exaggerates brain acidosis, particularly during recirculation. Hyperglycemia induced during ischemia does not change pH compared with the control group. Maintaining blood glucose concentration within the hypoglycemic range before ischemia significantly alleviates the degree of ischemic and recirculation acidosis. Unfortunately, using this model it is not possible to lower blood glucose concentrations rapidly enough during ischemia to estimate the influence of hypoglycemia induced after ischemia is induced (unpublished observations). Comparison of the NMR spectral intensity ratios between groups revealed no significant differences, implying that after 90 ± 8 minutes of recirculation these ratios are independent of the intracellular pH during ischemia and during initial recirculation and is consistent with the findings of Ljunggren et al [23] of a constant metabolic energy charge independent of preischemic blood glucose manipulation.

Caution must be exercised in relating our results of transient global cerebral ischemia, which is more relevant to cardiac arrest, to the human stroke condition of long-standing focal ischemia. However, as an indication of the similarity between significant differences within or among groups at baseline or after 90 minutes of recirculation.

During ischemia, pH of the control and hyperglycemic before groups were similar, with a tendency toward lower (but not significantly so) pH in the former than the latter group at the third and fourth measurements; however, the hypoglycemic group exhibited significantly less acidosis, that is, higher pH, than either group (p < 0.007) then.

Changes in pH during the first 12 minutes of recirculation were different for the control, hyperglycemic before, and hypoglycemic groups (p < 0.001). The hypoglycemic group consistently had a significantly higher pH during initial recirculation than the control or hyperglycemic before groups (p < 0.001). The hyperglycemic before group exhibited a significant decline in pH between 4 and 8 minutes of recirculation from that during ischemia (p < 0.003). A prolongation of tissue acidosis was noted in all groups except the hypoglycemic group.

The hyperglycemic before group exhibited a consistently lower pH during both ischemia and initial recirculation than the hyperglycemic during group (p < 0.02, p < 0.001, p < 0.01 at the first three recirculation measurements, respectively). No significant differences in pH were found between the control and hyperglycemic during groups during either ischemia or recirculation.
global animal models and focal human ischemia, we recently reported the case of a hyperglycemic patient with a large ischemic stroke studied serially using $^{31}$P NMR. We found persistent hyperglycemia to be associated with prolonged acidosis in the ischemic brain and concomitant failure of high-energy phosphates to recover. This in vivo human data supports the association of hyperglycemia with low brain pH and poor clinical outcome. Further studies on the regulation of blood glucose concentration on cerebral high-energy phosphate metabolism in focal ischemia are clearly warranted.

In attempting to understand the mechanisms whereby different pH values occur among groups, tissue lactate concentrations must be considered. However, the relation of lactate concentration to pH is not straightforward, and that between glucose and pH is complex. A direct couple of cerebral tissue pH and lactate concentration appears inconsistent with our data of pH during initial recirculation. All our groups exhibited a gradual return to baseline beginning 8–12 minutes after recirculation. However, there were distinct differences among groups, inasmuch as the hyperglycemic before group exhibited a transient severe further decline in pH between 4 and 8 minutes after recirculation. There is no immediate explanation for this observation. The decline in intracellular pH does not correlate with any known transient increase in lactate concentration, and the time course of tissue lactate concentration after ischemia does not match that of pH. A dissociation between brain lactate concentration and tissue pH has been recently demonstrated in microelectrode measurements of tissue pH and CO$_2$ in ischemic brain during in vitro measurements of pH and lactate concentration in reversible near-complete cerebral ischemia, in experimental brain tumors, and in vivo studies by Kraig et al. The transient decline in intracellular pH cannot be attributed to reperfusion with glucose-rich blood at a time of limited aerobic metabolic activity since the hyperglycemic during group failed to exhibit the same effect, even though recirculating blood has a greater glucose content. Likewise, based on previous studies in a similar model of global cerebral ischemia, postischemic cerebral blood flow did not significantly differ between hyperglycemic before, control (normoglycemic), and hypoglycemic groups and therefore is an unlikely explanation for these pH findings.

The differences in pH among groups appear to depend on preischemic brain glucose stores. Intravascular pH measurements during global ischemia in vivo have demonstrated that hyperglycemic conditions are associated with a more rapid decline in intracellular pH, while hypoglycemic conditions are associated with a more gradual return to baseline. It is plausible that these findings reflect differences in the regulation of cerebral pH during ischemia, with hyperglycemic conditions promoting a more rapid decline in intracellular pH, while hypoglycemic conditions promote a more gradual return.

The significance of these findings is that they offer insights into the mechanisms underlying the regulation of cerebral pH during ischemia, and may have implications for the development of therapeutic strategies to improve cerebral outcome after ischemic injury.
cellular glucose concentration falls to 0 when blood glucose concentration falls to <3 mmol/l; above this level intracellular concentrations vary with blood concentrations.27,28 Our hypoglycemic cats had maximum blood glucose concentrations of 2.2 mmol/l before ischemia and thus negligible tissue glucose stores. The control and hyperglycemic during groups exhibited nearly identical pH values during ischemia and reperfusion. In both groups the preischemic and residual (at the termination of ischemia) glucose stores should be identical. The hyperglycemic before group had elevated tissue glucose concentrations before ischemia as well as elevated glucose and glycogen concentrations during ischemia.23 Thus, the common factor that varies (inversely) with initial recirculation pH is the preischemic blood glucose concentration. How preischemic cerebral glucose stores link to reperfusion acidosis remains unclear.

Acknowledgments

The authors gratefully acknowledge Dr. Jay Gorell, Dr. Susan Fagan (technical assistance), and Mary Rexroad (manuscript preparation).

References


Key Words: cerebral ischemia • hyperglycemia • hypoglycemia • cats
Global cerebral ischemia and intracellular pH during hyperglycemia and hypoglycemia in cats.

M Chopp, K M Welch, C D Tidwell and J A Helpern

Stroke. 1988;19:1383-1387
doi: 10.1161/01.STR.19.11.1383

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/19/11/1383

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/