Effect of Mild Hyperthermia on Recovery of Metabolic Function After Global Cerebral Ischemia in Cats

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

We investigated the effect of mild whole-body hyperthermia before and after 16 minutes of global cerebral ischemia on metabolic recovery during recirculation in cats using in vivo phosphorus-31 nuclear magnetic resonance spectroscopy. Hyperthermia (temperature 40.6±0.2° C) was induced ≥1 hour before ischemia and was maintained during 1.5–2 hours of recirculation in nine cats; four cats were subjected to hyperthermia without cerebral ischemia, six to hyperthermia during recirculation (after return of intracellular pH to preischemic values), and 14 to normothermic ischemia and recirculation. Our data indicate that preischemic hyperthermia results in an intracellular cerebral pH during recirculation significantly lower than that in normothermic cats. In hyperthermic cats β-ATP and phosphocreatine (PCr) concentrations and the ratio of PCr to inorganic phosphate failed to return to preischemic levels during recirculation in contrast to normothermic cats. Hyperthermia without ischemia and hyperthermia during recirculation had no significant effect on intracellular pH. Thus, preischemic hyperthermia has a detrimental effect on metabolic recovery after transient global cerebral ischemia. (Stroke 1988;19:1521-1525)

Fever or subfebrility in stroke patients causes deterioration of neurologic function by mechanisms as yet unknown.1 In normal brain, body temperature increases of 1–2° C over a period of hours in response to infection or even ambient thermal conditions have no significant detrimental effects on cerebral metabolism.2 The effects of similar hyperthermia (temperature of ≤41° C) on cerebral high-energy phosphate metabolism and intracellular pH has not been addressed under conditions of cerebral ischemia. Accordingly, we used in vivo phosphorus-31 nuclear magnetic resonance (31P-NMR) spectroscopy to measure relative concentrations of high-energy phosphate metabolites and intracellular pH before, during, and after transient global cerebral ischemia in cats subsequent to mild whole-body hyperthermia. Our data suggest that preischemic mild hyperthermia prolongs ischemic brain acidosis and has a detrimental effect on metabolic recovery from transient global cerebral ischemia.

Materials and Methods

We used 38 conditioned cats weighing 2.2–3.6 kg. Surgical preparation and induction of transient (16 minutes) global cerebral ischemia using a combination of systemic arterial hypotension and inflation of a cervical cuff were identical to those previously reported.3 Each cat was placed on a water blanket. A water bath recirculator-heater (Model E-12, Haake, Berlin, FRG) connected to a feedback-regulated temperature controller (YSI model 73-A, Yellow Springs Instrument Company, Yellow Springs, Ohio) was used to regulate the cat’s temperature. Temperature probes were placed rectally and in the abdominal wall. Over 1–1.5 hours the rectal temperature of cats made hyperthermic rose to between 40° and 41° C. Temperature gradients between rectal and subcutaneous temperatures were kept to <0.2° C.

The time course and relations between cerebral, rectal, esophageal, and subcutaneous temperatures were measured in five cats: two cats made hyperthermic before ischemia, two cats subjected to normothermic ischemia, and one cat made hyperthermic without being subjected to ischemia. We used two copper constantan thermocouples (100
µm in diameter) in each cat to measure cerebral thermal distribution 1 and 2 cm, respectively, posterior to the bregma and 1 cm left of the midline. A small burr hole was made through the skull; the dura was punctured with a 20-gauge needle that also served as a guide for the thermocouples. The thermocouples were inserted 1 cm beneath the surface of the skull, and the needles were removed. Temperature was measured every 2 minutes. These five cats were not subjected to 31P-NMR spectroscopy but were subjected to surgical procedures and experimental protocols identical to those of cats in which 31P-NMR spectroscopy was performed.

A 1.89-T superconducting 60-cm-bore magnet with a Bruker Biospec console was used for in vivo 31P-NMR spectroscopy. A 2.5-cm, two-turn double-tuned surface coil (proton and phosphorus resonance) was placed over the parietal cortex of each cat with the skull intact. To avoid contamination of the spectra, all muscle within 3 cm of the coil was cleared. 31P-NMR spectra were obtained using a 40-µsec effective 90° pulse applied to the coil. Four hundred transients were averaged over each 4-minute interval. Spectral width was 4,000 Hz.

Four control spectra were obtained before heating in each cat to be made hyperthermic. An additional four spectra were obtained after heating before ischemia. Spectra were collected at 4-minute intervals during ischemia and during the approximately 96 minutes of recirculation. The recirculation period was predicated on our observation that in normothermic animals β-adenosine 5'-triphosphate (β-ATP) and phosphocreatine (PCr) concentrations return to preischemic values within the first 32 minutes of recirculation. Therefore, 1.5–2.0 hours of recirculation was deemed adequate to compare metabolic return in hyperthermic with normothermic groups. We calculated control parameters as the ratios of β-ATP, PCr, inorganic phosphate (Pi), PCr/Pi, and PCr/β-ATP peak heights to control peak heights. Intracellular pH was determined at 4-minute intervals by the chemical shift of Pi from PCr. Spectra were processed with a profile-correction routine supplied by the manufacturer and 10-Hz exponential line broadening.

We studied four groups of cats. Nine cats were made hyperthermic and the hyperthermia was maintained (±0.1°C) for ≥1 hour before ischemia, during ischemia, and during recirculation. In six cats, hyperthermia was initiated after 1–1.5 hours of recirculation, when intracellular pH returned to control values; hyperthermia was maintained and these six cats were monitored for 2–4 hours after reaching steady-state temperature. Technical difficulties associated with our method of heating the cats precluded the induction of steady-state hyperthermia during recirculation before the return of pH to control values. Fourteen normothermic (temperature between 38.5° and 39.2°C) cats were subject to ischemia and recirculation; data from seven of these normothermic cats have been reported.3 The effect of hyperthermia on cerebral energy metabolism without cerebral ischemia was measured in four cats made hyperthermic over 1–1.5 hours and maintained at a constant temperature (40.8±0.1°C) for 3–5 more hours.

Profile analysis was performed on all 31P-NMR and physiologic measures. Only six recirculation spectra (4, 16, 32, 48, 64, and 96 minutes) were analyzed; for each, a mean value of the 31P-NMR parameter was calculated from the time of interest and the times immediately before and immediately after (except for the 4-minute time, which was averaged only with the 16-minute time). Physiologic data were obtained before ischemia, before and after hyperthermia, and four times during recirculation (16, 32, 64, and 96 minutes). If the group effect or the group x time interaction were found to be significant in the profile analysis, subanalyses (t tests) were performed with Bonferroni’s multiple comparison adjustment made for each subanalysis. 31P-NMR and physiologic values at all points of interest within each group were compared with control values using paired t tests.

### Results

Table 1 shows the physiologic data for the normothermic and hyperthermic groups. No significant differences between groups were found in pH, PCO₂, PO₂, and serum glucose concentration except for pH

### Table 1. Physiologic Data for Cats

<table>
<thead>
<tr>
<th>Time</th>
<th>pH</th>
<th>PCO₂ (mm Hg)</th>
<th>PO₂ (mm Hg)</th>
<th>MABP (mm Hg)</th>
<th>SAG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.34±0.06</td>
<td>31.7±2.4</td>
<td>142±22</td>
<td>114±10</td>
<td>145±34</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>7.33±0.04</td>
<td>32.9±4.0</td>
<td>131±28</td>
<td>111±11</td>
<td>176±24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(Re)circulation</th>
<th>pH</th>
<th>PCO₂ (mm Hg)</th>
<th>PO₂ (mm Hg)</th>
<th>MABP (mm Hg)</th>
<th>SAG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 min</td>
<td>7.17±0.08*</td>
<td>40.7±12.9</td>
<td>122±32*</td>
<td>111±17</td>
<td>126±31</td>
</tr>
<tr>
<td>32 min</td>
<td>7.16±0.06*</td>
<td>37.1±6.5</td>
<td>150±53</td>
<td>113±7</td>
<td>204±77</td>
</tr>
<tr>
<td>64 min</td>
<td>7.18±0.08*</td>
<td>36.3±8.1</td>
<td>127±11</td>
<td>105±15</td>
<td>190±77</td>
</tr>
<tr>
<td>96 min</td>
<td>7.24±0.15</td>
<td>33.4±10.4</td>
<td>129±30</td>
<td>105±20</td>
<td>142±67</td>
</tr>
</tbody>
</table>

Data are mean±SD. MABP, mean arterial blood pressure; SAG, serum arterial glucose concentration.

*p<0.01 different from control.

†p<0.006, p<0.009 different from normothermia.
at 96 minutes of recirculation ($p<0.009$) and $Po_2$ at 64 minutes of recirculation ($p<0.006$). Comparison of recirculation values with control values within each group showed that arterial pH remained aci-

dotic throughout recirculation for the hyperthermic group. Except for a 16-minute $Po_2$ in the normo-

thermic group, there were no significant differences 

within the groups for any other physiologic measure 

during recirculation compared with control values. 

There were also no significant differences in the 

hyperthermic group for physiologic parameters after 

heating compared with control values. 

Figure 1 shows $^3$P-NMR spectra from repre-

sentative normothermic and preischemia hyperther-

mic cats before ischemia (control), during ischemia, 

and during recirculation. In the preischemia hyper-

thermic group, $\beta$-ATP concentration declined 

approximately 4.7% from control values ($p<0.02$) 

after heating, before ischemia; there were no changes 

observed in other peak heights with heating. Both 

normothermic and preischemia hyperthermic cats 

exhibited a rapid decline in adenylate peaks to noise 

levels during ischemia. During recirculation, spec-

tra from the normothermic group returned to con-

trol values; in contrast, the preischemia hyperther-

mic cats failed to regain control values. Table 2 
presents $\beta$-ATP, PCr, Pi, PCr/Pi, and PCr/$\beta$-ATP 

contents during six recirculation time points as 

fractions of control values. The data suggest a 

failure in the preischemia hyperthermic group of the 

adenylate peaks to return to control values. 

Figure 2 shows the time course of intracellular 
P pH for the normothermic and preischemia hyper-

thermic groups. The normothermic group exhibited a 

transient 4–8-minute prolongation of intracellular acido-

sis during recirculation, with a return to >95% 
of the control pH value within 40 minutes. In the 

preischemia hyperthermic group, intracellular pH 

remained acidic during recirculation. Profile analy-

sis revealed a marginally nonsignificant group $\times$

time interaction for pH ($p<0.07$). $t$ tests revealed 

significantly lower pH for the hyperthermic than for 

the normothermic group after 32 and 48 minutes of 

recirculation ($p<0.0005$ and $p<0.0006$, re-

spectively); immediately upon recirculation, pH values 

were not significantly different. At the other recir-

culation times analyzed, pH values for the preis-

chemia hyperthermic group were lower than for the 

normothermic group ($0.02<p<0.05$). 

The four no-ischemia hyperthermic cats failed to 

exhibit intracellular tissue acidosis or declines in 

high-energy phosphate peak heights or ratios. Intra-

cellular pH and adenylate peak heights were also 

unaltered by recirculation hyperthermia. 

Figure 3 shows the time course of cerebral and 

rectal temperatures for a representative preis-

chemia hyperthermic cat. Brain and rectal tempera-

tures in the two preischemia hyperthermic cats rose 
in unison. Esophageal, subcutaneous (not shown), 

and rectal temperatures remained within 0.2° C of 

each other throughout the experiment. Cerebral 

temperature dropped precipitously during ischemia 

(3.8±0.1° C). During recirculation, brain tempera-
ture increased rapidly and achieved a steady state 

approximately 0.3° C below rectal temperature. 

Discussion 

Our data indicate that mild hyperthermia induced 

before transient global cerebral ischemia causes 

severe tissue acidosis that is prolonged during recir-
culation and that concentrations of high-energy 

phosphate metabolites fail to return to control lev-

els. Measurements of brain temperature versus core 
temperature revealed that the average brain temper-

ture was likely no more than 1–2° C above normal 

body temperature for cats (38.5–39.2° C) during
TABLE 2. Metabolic Recovery After 16-Minute Transient Global Cerebral Ischemia in Cats Measured by Phosphorus-
31 Nuclear Magnetic Resonance Spectroscopy

<table>
<thead>
<tr>
<th>Time after recirculation (min)</th>
<th>β-ATP</th>
<th>PCr</th>
<th>Pi</th>
<th>PCr/Pi</th>
<th>PCr/β-ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermia (n=14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.73±0.12*</td>
<td>0.68±0.07*</td>
<td>2.89±0.30*</td>
<td>0.26±0.04*</td>
<td>0.96±0.18</td>
</tr>
<tr>
<td>16</td>
<td>0.88±0.15*</td>
<td>0.87±0.03*</td>
<td>1.71±0.26*</td>
<td>0.53±0.10*</td>
<td>1.00±0.18</td>
</tr>
<tr>
<td>32</td>
<td>0.93±0.07</td>
<td>0.97±0.04</td>
<td>1.27±0.18</td>
<td>0.78±0.12*</td>
<td>1.04±0.11</td>
</tr>
<tr>
<td>48</td>
<td>0.91±0.11</td>
<td>1.03±0.07</td>
<td>1.23±0.26</td>
<td>0.88±0.18</td>
<td>1.14±0.13</td>
</tr>
<tr>
<td>64</td>
<td>0.87±0.04*</td>
<td>0.97±0.06</td>
<td>1.11±0.08</td>
<td>0.89±0.10</td>
<td>1.12±0.10</td>
</tr>
<tr>
<td>96</td>
<td>0.93±0.06</td>
<td>0.96±0.06</td>
<td>1.21±0.14</td>
<td>0.82±0.15</td>
<td>1.02±0.10</td>
</tr>
<tr>
<td>Preischemic hyperthermia (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.61±0.13*</td>
<td>0.59±0.10</td>
<td>4.62±1.29*</td>
<td>0.14±0.05*</td>
<td>1.00±0.28</td>
</tr>
<tr>
<td>16</td>
<td>0.73±0.11*</td>
<td>0.72±0.10†</td>
<td>3.33±1.13†</td>
<td>0.24±0.09†</td>
<td>1.00±0.18</td>
</tr>
<tr>
<td>32</td>
<td>0.81±0.10*</td>
<td>0.78±0.09†</td>
<td>2.57±0.95*</td>
<td>0.36±0.20†</td>
<td>0.99±0.14</td>
</tr>
<tr>
<td>48</td>
<td>0.83±0.09*</td>
<td>0.83±0.14†</td>
<td>1.92±0.89</td>
<td>0.55±0.29*</td>
<td>1.00±0.17</td>
</tr>
<tr>
<td>64</td>
<td>0.85±0.11*</td>
<td>0.81±0.17</td>
<td>1.78±0.94</td>
<td>0.59±0.35</td>
<td>0.96±0.16</td>
</tr>
<tr>
<td>96</td>
<td>0.74±0.09†</td>
<td>0.72±0.16†</td>
<td>2.19±1.28</td>
<td>0.49±0.34*</td>
<td>0.98±0.18</td>
</tr>
</tbody>
</table>

Data are mean±SD ratio of peak height at indicated time to peak height before ischemia. β-ATP, β-adenosine 5′-triphosphate; PCr, phosphocreatine; Pi, inorganic phosphate.

*p<0.008 different from 1.0.
†p<0.008 different from normothermia.

hyperthermia. Although we did not measure the distribution of brain temperatures, particularly cortical temperature, it is unlikely that cortical temperatures were higher than those measured. Patterns of metabolic return during recirculation and ischemic neuronal damage have not been correlated. However, there is recent evidence relating biologic outcome, as measured by changes in electroencephalographic amplitudes, to the degree of metabolic dysfunction in middle cerebral artery occlusion in cats. Likewise, increased cerebral acidosis associated with hyperglycemia has been implicated to adversely affect the viability of cerebral tissue. Thus, hyperthermia and the associated failure to reestablish preschismic spectral values may lead to a worsened biologic outcome. The detrimental effects of hyperthermia on cerebral ischemia is also supported by a recent study by Busto et al in which small increments of intraschismic striatal brain temperature in rats were shown to accentuate cerebral histopathologic changes 3 days after ischemia. The implications of these findings for clinical stroke patients are profound since an increase in body and brain temperature of 1–2°C is not uncommon. The data also have important implications for the management of cardiac arrest patients, in whom

Figure 2. Graph of time course of mean±SD intracellular pH for 14 normothermic (●) and nine preischemic hyperthermic (○) cats. No significant differences are observed between groups before or during 16-minute global cerebral ischemia. However, during recirculation intracellular pH is significantly more acidic in preischemic hyperthermic than in normothermic group.

Figure 3. Graph of time course of temperature in representative preischemic hyperthermic cat. •, rectal temperature; □, brain temperature 1 cm posterior to bregma, 1 cm left of midline, 1 cm from skull surface; ○, brain temperature 2 cm posterior to bregma, 1 cm left of midline, 1 cm from skull surface. Note concurrent rectal and brain temperature changes before 16-minute global cerebral ischemia, decline of brain temperature during ischemia, and rapid return of brain temperature during recirculation.
aggressive efforts should be made to control body temperature to perhaps even subnormal levels.

The mechanisms whereby mild hyperthermia prolongs ischemic cerebral acidosis are unclear. Under nonischemic conditions in our study, hyperthermia did not cause changes in intracellular pH. The 4.7% decrease in \( \beta \)-ATP concentrations with hyperthermia before ischemia is consistent with data reported by Nilsson et al., though a significant decline was not reported in that study. A more recent study using microwave-induced cerebral hyperthermia reported 6.9% and 10.8% declines in ATP concentrations at temperatures elevated 1.4° and 3.4° C, respectively, in rats. Carlsson et al., using a heating bulb to induce hyperthermia, noted no change in cerebral ATP concentrations from normothermic values at 42° C in rats. The temperatures achieved in our experiments are therefore unlikely to cause direct metabolic damage. Hyperthermia stimulates the cerebral metabolic rate for oxygen (CMRO₂) and increases cerebral blood flow (CBF) 5–6%/° C in normal brain; an effect on CMRO₂ and CBF has not been studied during cerebral ischemia. Even if CMRO₂ were identical in ischemic and nonischemic hyperthermia, it is unlikely that hyperthermia would cause the degree of intracellular acidosis we observed. Furthermore, if the increased metabolic activity were totally anaerobic during recirculation, a proportional change in intracellular acidosis might be expected. Thus, for example, a 10% change in metabolic activity is inconsistent with the threefold increase in hydrogen ion concentration, that is, the pH change from 6.9 to 6.3 after 48 minutes of recirculation. Further studies are necessary to elucidate the mechanism by which hyperthermia prolongs postischemic brain acidosis and metabolic dysfunction.

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References

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