Reduction of Hyperthermic Ischemic Acidosis by a Conditioning Event in Cats

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We investigated the effects of multiple episodes of cerebral ischemia on intracellular brain pH using in vivo phosphorus-31 nuclear magnetic resonance spectroscopy. Four cats were subjected to two 16-minute episodes of complete global cerebral ischemia 6 hours apart; the second episode occurred under hyperthermic conditions (mean±SD body temperature 40.8±0.4°C). Intracellular pH in these four cats was compared with that in nine cats subjected to a single 16-minute episode of complete global cerebral ischemia under hyperthermic conditions (mean±SD body temperature 40.6±0.2°C). Intracellular pH during hyperthermic recirculation was significantly (p<0.03) greater in cats subjected to a previous ischemic event than that in cats subjected to only a single hyperthermic ischemic event. We speculate that the induction of heat shock proteins by an initial ischemic event may protect brain tissue from further ischemic insult. (Stroke 1989;20:1357-1360)

A n initial ischemic insult can protect tissue against subsequent ischemic insults. For example, brief intermittent episodes of myocardial ischemia limit infarct size and protect the myocardium from later ischemia.1 Protective cerebral ischemic conditioning, investigated by Mrsulja et al in gerbils, decreased the metabolic effect of a second ischemic insult administered ≥5 hours later. In previous work, we have reported that mild hyperthermia (40°-41°C) before transient complete global cerebral ischemia prolonged intracellular acidosis and inhibited the recovery of high-energy phosphates.3 We now report that when hyperthermia is preceded by transient complete global cerebral ischemia, this degraded metabolic response no longer occurs.

Materials and Methods

We used 17 conditioned cats weighing 2.2–3.6 kg, with surgical preparation and induction of complete global cerebral ischemia by a combination of systemic arterial hypotension and inflation of a cervical cuff as 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 (model 73-A, YSI, Yellow Springs, Ohio) was used to regulate temperature. Thermocouples were placed rectally and in the abdominal wall. Over 1–1.5 hours the cat’s rectal temperature rose to 40°–41°C. Temperature gradients between rectal and subcutaneous temperatures were kept to <0.2°C.

A 1.89-T superconducting 60-cm bore magnet with a Bruker Biospec console (Billerica, Massachusetts) was used for in vivo phosphorus-31 nuclear magnetic resonance (31P NMR) studies. A 2.5-cm two-turn double-tuned surface coil (proton and phosphorus resonance) was placed over the parietal cortex, with the skull intact. To eliminate contamination of the 31P NMR spectra, all scalp muscle within 3 cm of the coil was cleared. Spectra were obtained using a 40-μsec effective 90° pulse applied to the coil; 400 transients were averaged over 4 minutes, providing a repetition rate of 600 msec. Spectra were therefore partially saturated. Spectral width was 4,000 Hz. In all experimental protocols, spectra were continuously obtained before ischemia, during ischemia, and during recirculation. Intracellular pH was determined every 4 minutes by the chemical shift of inorganic phosphate from phosphocreatine.4,5 Spectra were processed with a profile-correction routine supplied by the manufacturer6 and 10-Hz exponential line broadening.

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For two episodes of ischemia, four cats were subjected to 16 minutes of normothermic complete global cerebral ischemia. After approximately 1 hour of normothermic recirculation, each cat was heated. The animal’s mean ± SD rectal temperature slowly increased to 40.8 ± 0.4°C over 1.5 hours and hyperthermia was maintained for an additional 3.5 hours. A 16-minute hyperthermic ischemic insult was then induced, and hyperthermia was maintained for approximately 1.5–2 hours of recirculation.

For the single episode of ischemia, nine cats were heated to a mean ± SD rectal temperature of 40.6 ± 0.2°C over 1.5 hours and hyperthermia was maintained for 1.5 hours before ischemia, during 16 minutes of ischemia, and during 1.5–2 hours of recirculation. Data from these nine cats have been reported.3

As controls (to determine if >1.5 hours of pre-ischemic steady-state hyperthermia prolongs post-ischemic brain tissue acidosis as for 1.5-hour pre-ischemic steady-state hyperthermia), two cats were heated over 1.5 hours and steady-state hyperthermia (40.7 ± 0.2°C) was maintained for 3.5 hours. These two control cats were then subjected to 16 minutes of hyperthermic ischemia and 1.5–2 hours of hyperthermic recirculation.

Repeated-measures analysis of variance (ANOVA) was performed on the average of four preischemia (baseline) intracellular brain pH values, four intracellular brain pH values obtained during ischemia, and values obtained after 4, 16, 32, 48, and 60 minutes of recirculation. Analysis of intracellular brain pH data from all 13 cats necessitated restriction of data to these times; data after >60 minutes of recirculation were absent from one or more cats due to our inability to either detect or resolve the inorganic phosphate resonance. Repeated-measures ANOVA was also performed on systemic physiologic data (serum arterial pH, Pao2, Paco2, mean arterial blood pressure, and serum arterial glucose concentration) obtained before ischemia and after 16, 32, 64, and 96 minutes of recirculation. Repeated-measures ANOVA was used to compare the four cats subjected to two episodes of ischemia and to compare the data obtained during the second recirculation period in these four cats with that obtained in the nine cats subjected to a single episode of ischemia.

To investigate the possible induction of newly-synthesized proteins in the brain, we also performed one-dimensional gel electrophoresis on two additional cats, pulse-labeling the brains with [35S]methionine 2 hours after killing the cat.7 Brain tissue selected for labeling came from the parietal cortex and corresponded to tissue sampled by 31P NMR.

### Results

Table 1 shows the systemic physiologic data for the two experimental groups. Among the four cats...
subjected to two episodes of ischemia, differences in mean arterial blood pressure were detected between the first and second recirculation periods ($p<0.03$); a paired $t$ test of the data for corresponding times, however, revealed no significance. A paired $t$ test of the data after 96 minutes of recirculation revealed a significant difference ($p<0.009$) in serum arterial glucose concentration between the first and second recirculation periods. No other significant differences in physiologic data or in intracellular brain pH (data not shown) for the matched times were detected.

We also compared the data obtained before, during, and after the second ischemic episode with that obtained at matched times before, during, and after the single ischemic episode. Figure 1 is a plot of the intracellular brain pH for this comparison. A significant ($p<0.03$) group difference was detected; during recirculation, intracellular brain pH was lower in the group subjected to a single episode of ischemia.

Control cats in which a single episode of ischemia was preceded by 3.5 hours of steady-state hyperthermia displayed prolonged intracellular acidosis during recirculation similar to that in the nine cats subjected to 1.5 hours of steady-state hyperthermia prior to a single episode of ischemia. In one of the two control cats, intracellular brain pH remained near 6.0 during the entire recirculation period; intracellular pH in the other control cat returned to baseline after 76 minutes of recirculation.

Figure 2 shows two lanes of one-dimensional gel electrophoresis for in vitro pulse-labeling of newly synthesized proteins in brain with $[35S]$methionine 2 hours after sacrifice. A control cat not subjected to ischemia shows little 70-kDa HSP. In contrast, the control cat subjected to ischemia that survived 2 hours exhibited 70-kDa HSP.

**Discussion**

Our data indicate that when a cerebral ischemic episode under hyperthermic conditions is preceded (6 hours) by a previous ischemic episode, intracellular brain pH is significantly greater than when a hyperthermic ischemic insult is not preceded by an ischemic event. Thus, the first ischemic event evokes a metabolic protection against subsequent hyperthermic ischemia.
The lessening severity of metabolic abnormality associated with repeated ischemic events is analogous to the phenomenon of thermotolerance,8-11 in which cells and organisms develop resistance to subsequent hyperthermia after an initial heat treatment. Thermotolerant cells can sustain glucose deprivation longer than control cells.12 Likewise, there is an increased ability of thermotolerant cells to maintain intracellular levels of adenosine triphosphate when challenged by hyperthermia.13 Acquisition of thermotolerance may be compared with the preferential synthesis of heat shock proteins (HSPs), particularly that of the 70-kDa HSP.14-19 We speculate that the metabolic protection we observed after a second ischemic episode is associated with the induction of HSP. Brain tissue perturbed by ischemia generates HSPs identical to those generated by hyperthermia, trauma, and metabolic injury.7,20-23 The HSPs induced by ischemia may have the same homeostatic function (protection of cells from thermal and metabolic injury) as those induced by hyperthermia. We performed one-dimensional gel electrophoresis to determine if HSPs were induced in cats subjected to cerebral ischemia.

Because we found an increase in HSP after ischemia, albeit in only one cat, further studies documenting the accumulation as well as the rate of HSP synthesis under conditions of experimental ischemia are necessary. However, our HSP pulse-labeling experiment simply illustrates the ability of an ischemic insult to evoke HSP synthesis in cats and thus supports our speculation concerning the role of HSP in ischemic metabolic protection.

Our hypothesis of the protective effects of HSP is also supported by a recent study of high-intensity light damage to rat retina.19 Barbe et al19 found that when HSPs are induced before the retinal insult by a transient thermal shock, the retina is protected against cell damage from high-intensity light. This study also suggests that transient hyperthermic shock protects brain from ischemic insult.

In summary, we found an initial cerebral ischemic event to reduce intracellular acidosis caused by a subsequent hyperthermic ischemic event. Our preliminary data suggest that this metabolic protection is associated with the induction of HSP by the first ischemic insult.

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