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## The Relation between Cerebral Metabolic Rate and Ischemic Depolarization: A Comparison of the Effects of Hypothermia, Pentobarbital, and Isoflurane

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## Abstract

Background: Reductions in cerebral metabolic rate may increase the brain's tolerance of ischemia. However, outcome studies suggest that reductions in cerebral metabolic rate produced by anesthetics and by hypothermia may not be equally efficacious. To examine this question, we measured the effects of hypothermia, pentobarbital, and isoflurane on the cerebral metabolic rate for glucose ($CMR_G$) and on the time to the loss of normal membrane ion gradients (terminal ischemic depolarization) of the cortex during complete global ischemia.

Methods: As pericranial temperature was varied between 39 and 25 degrees Celsius in normocapnic halothane-anesthetized rats, $CMR_G$ (using $^{14}$ Carbon-deoxyglucose)

## Graphics



or the time to depolarization (using a glass microelectrode in the cortex) after a Potassium sup + -induced cardiac arrest was measured. In other studies, $CMR_G$ and depolarization times were measured in normothermic animals (37.7 plus/minus 0.2 degree Celsius) anesthetized with high-dose pentobarbital or isoflurane (both producing burst suppression on the electroencephalogram) or in halothane-anesthetized animals whose temperatures were reduced to 27.4 plus/minus 0.3 degree Celsius. These three states were designed to produce equivalent $CMR_G$ values.

Results: As temperature was reduced from 39 to 25 degrees Celsius, $CMR_G$ decreased from 66 to 21 micro Meter *symbol* 100 g sup -1 *symbol* min$^1$ (Q10 = 2.30), and depolarization times increased from 76 to 326 s. In similarly anesthetized animals at approximately 27 degrees Celsius, $CMR_G$ was 32 plus/minus 4 micro Meter *symbol* 100 g sup -1 *symbol* min sup -1 (mean plus/minus SD), whereas in normothermic pentobarbital- and isoflurane-anesthetized rats, $CMR_G$ values were 33 plus/minus 3 and 37 plus/minus 4 micro Meter *symbol* 100 g$^1$ *symbol* min sup -1, respectively (P = 0.072 by one-way analysis of variance). Despite these similar metabolic rates, the times to depolarization were markedly different: for hypothermia it was 253 plus/minus 29 s, for pentobarbital 109 plus/minus 24 s, and for isoflurane 130 plus/minus 28 s (P < 0.0001).

Conclusions: The time to terminal depolarization is believed to be a measure of the rate at which energy stores are depleted. In this study there was a strong correlation between hypothermic reductions in $CMR_G$ and increases in the time to depolarization. This finding supports the belief that metabolic suppression may offer some cerebral protection. However, equivalent reductions in $CMR_G$ produced by hypothermia and by anesthesia were not equivalent in their effects on membrane failure. Whether hypothermia slows energy depletion by some unique mechanism or directly retards depolarization is unknown.

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Key words: Anesthetics, intravenous: pentobarbital. Anesthetics, volatile: isoflurane. Brain: ischemic depolarization; metabolic rate. Measurement techniques: cerebral metabolic rate for glucose. Temperature: hypothermia.

THERE are many mechanisms by which the brain can be protected against ischemic injury. Perhaps the oldest hypothesis is that a reduction in metabolic demand should increase tolerance to a decrease in oxygen and nutrient supply and therefore that the protective efficacy of hypothermia and of some anesthetics is related to reductions in cerebral metabolic rate. However, studies suggest that this is an incomplete explanation. [1-6] For example, Busto et al. showed that cerebral injury varied sigmoidally as temperature decreased--a pattern that did not parallel changes in the brain's concentration of high-energy phosphates or in predicted cerebral metabolic rate. [2] In addition, Sano et al. demonstrated that mild hypothermia (35 degrees Celsius) afforded a greater degree of histopathologic protection against forebrain ischemia than did isoflurane. [7].

Metabolic suppression is thought to protect the brain by slowing the depletion of high-energy phosphates. Occurring during the first seconds after the onset of ischemia is a complex series of ionic events that may not be dependent on adenosine

triphosphate (ATP). [8] Eventually, however, when ATP is critically depleted, normal transmembrane ion gradients are lost, [9-12] and there is sudden depolarization of the neuron. This change is seen as a sudden increase in extracellular Potassium sup + concentration or as a sudden shift in the cortical direct-current potential. Depolarization triggers a cascade of biochemical events that play an important role in cell damage. [13,14] It is hence reasonable to conclude that any intervention that delays the time to depolarization may be protective. Astrup et al. suggested that there is a direct relation between cerebral metabolic rate for oxygen ($CMR_{O2}$) and the time to membrane depolarization after circulatory arrest. [9] In an earlier experiment in our laboratory, animals anesthetized with isoflurane/nitrous oxide had a lower cerebral metabolic rate for glucose ($CMR_G$) and a longer time to terminal depolarization than did animals anesthetized with halothane/nitrous oxide. [15] However, although this result is consistent with the metabolic protection hypothesis, Astrup et al. noted that hypothermic reductions in $CMR_{O2}$ seemed to delay depolarization more than did barbiturate-induced metabolic suppression. [16] This finding suggests that hypothermia and anesthetics have different effects on the relation between cerebral metabolism and the rate of ischemic membrane failure.

To investigate this difference, we directly examined the relation among temperature, $CMR_G$, and the times to cortical depolarization after a cardiac arrest (complete global ischemia). We then compared the times to depolarization in animals in which equivalent reductions in $CMR_G$ were produced by hypothermia or by high-dose barbiturate and isoflurane anesthesia.

## Materials and Methods

All aspects of this study were approved by the University of Iowa Animal Care and Use Committee. Animals in each of the experiments were randomly assigned to the various study groups.

### Part I: Hypothermia, Terminal Depolarization, and Cerebral Metabolic Rate for Glucose

Our first goal was to examine the relations among temperature, time to terminal ischemic depolarization, and $CMR_G$. These experiments were performed in two groups of animals, with depolarization intervals examined in one and $CMR_G$ measured in the other.

Male Sprague-Dawley rats weighing 325-350 g, were used. Animals were allowed free access to food and water until anesthetized. Anesthesia was induced with 4% halothane in oxygen in a closed plastic box. After tissue infiltration with 1% lidocaine, a tracheotomy was performed and mechanical ventilation was started with an inspired gas mixture of 1.5% halothane in oxygen, using a tidal volume of 2.5-3.5 ml and a rate of 32-45 breaths/min. A femoral artery and vein were cannulated after lidocaine infiltration. The arterial and venous catheters were used for continuous blood pressure monitoring and arterial blood sampling, and for the infusion of fluids and drugs respectively. Muscle relaxation was achieved with 0.2 mg intravenous pancuronium given as needed. The animal was then turned prone and the head fixed in a stereotactic frame. The scalp was infiltrated with 1% lidocaine and reflected laterally to expose the calvarium. A 2 X 2 mm left parietal craniectomy (located [nearly equal] 2 mm caudal to the bregma and 2 mm lateral to the midline) was drilled. This was done under a microscope, and the drilling site was irrigated with cool saline to avoid thermal trauma. The dura was left intact. When surgery was complete, the inspired halothane concentration was reduced to approximately 0.8% as verified with an

infrared analyzer, and 50% $N_2$ O was added to the inspired gas mixture. A continuous infusion of lactated Ringer's solution was started at the rate of 1 ml/h, and supplemental 1-3 ml doses of 6% hetastarch were given to maintain mean arterial pressure (MAP) greater than 80 mmHg if necessary. Platinum needle electrodes were inserted into the temporalis muscles bilaterally to permit the recording of a single biparietal electroencephalogram (EEG). Temperature was recorded in each animal in two locations: a needle thermistor (524, Yellow Springs Instruments, Yellow Springs, OH) was placed into the pericranial tissue adjacent to the craniotomy, and a probe (401, Yellow Springs Instruments) was inserted rectally.

Hematocrit, pH, and arterial carbon dioxide and oxygen tensions were measured intermittently and ventilation was adjusted if necessary to maintain arterial oxygen tension less than 100 mmHg and arterial carbon dioxide tension between 35 and 42 mmHg. All blood gas values were measured at 38 degrees Celsius and reported without temperature correction (alpha-stat management).

When preparation was complete, pericranial temperature was adjusted to and maintained at target values of 41, 39, 38, 37, 35, 32, 30, 28, or 25 degrees Celsius using a warming blanket in normothermic and hyperthermic animals or a ice-water/perfused water-jacket placed around animal's body in hypothermic animals. One to three rats were studied at each temperature; Figure 1 shows the distribution of animals among temperature groups. Fifty units of heparin was administered intravenously to all rats.

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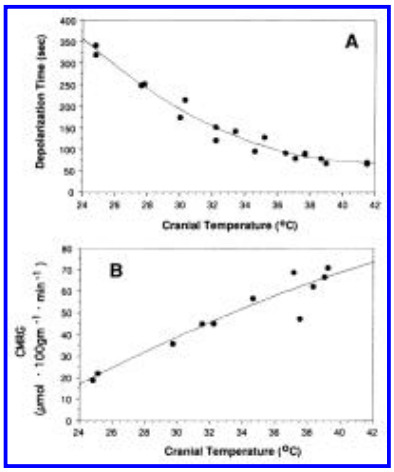


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Figure 1. (A) Plot of pericranial temperature versus depolarization times in part I. (B) Pericranial temperature versus cortical cerebral metabolic rate for glucose ($CMR_G$). In both panels, the fitted lines are derived from a second-order polynomial. The calculated ratio of metabolic rates over a temperature interval of 10 degrees Celsius ($Q_{10}$) for temperature versus depolarization was 2.83; for temperature versus $CMR_G$, the $Q_{10}$ was 2.30.

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Terminal Depolarization. Nineteen animals were used to examine the relation between temperature and the time from an induced cardiac arrest to the onset of terminal cortical depolarization. One to three rats were studied at each of the above pericranial temperatures. In each, a small slit was made in the dura, and a micromanipulator was used to insert a saline-filled glass micropipette (tip diameter of [nearly equal] 5 micro meter) 0.5 mm into the cortex. Care was taken to avoid damage to cortical vessels. An Silver-AgCl wire in the barrel served as the electric contact and a Silver-AgCl rod was inserted into the dorsal neck muscles as the reference. The direct-current potential between these electrodes was measured with an amplifier (7P122, Grass Instruments, Quincy, MA) equipped with a high-impedance input probe (H1P5, Grass). After a stabilization period of 50-80 min (with at least 30 min at a stable target temperature) the animals were killed with 0.5

ml saturated KCl given intravenously. The subsequent time (in seconds) to depolarization was recorded (timed from the onset of the cardiac arrest).

Measurement of Cerebral Metabolic Rate for Glucose. Eleven rats were used to examine the relation between temperature and $CMR_G$. Animals were prepared as above, except that bilateral femoral arterial and venous catheters were placed and no microelectrode was placed after the craniectomy. One to three animals were studied at each of the temperatures noted above. After a stabilization period of 30-50 min, 35 micro Ci/kg [sup 14 Carbon]-deoxyglucose [sup 14 Carbon]2-DG) (ARC-111A, American Radiolabeled Chemicals, St. Louis, MO) was infused intravenously over a 45-s period. Arterial blood samples, 150-200 micro liter, were taken 0.5, 1, 2, 3, 5, 7, 10, 15, 30, and 45 min after ending the infusion, while MAP was kept greater than 80 mmHg by infusion of 6% hetastarch or rat donor blood if necessary. At the end of the 45- min sampling period, the animals were decapitated; the brains were removed; and cortical gray matter from the area underlying the craniectomy was dissected free. This tissue was placed on a glass coverslip, weighed, and placed in a 20-ml scintillation vial (sample weight [nearly equal] 100 mg). One milliliter tissue solubilizer (TS-1, Research Products International, Mt. Prospect, IL) was added, the vial was capped and was heated overnight at 50 degrees Celsius. The sample was neutralized with 34 micro liter glacial acetic acid, and 16 ml scintillation cocktail (3a70B, Research Products International) was added. The blood samples were centrifuged, and glucose concentrations were measured in 10 micro liter separated plasma (model 27 analyzer, Yellow Springs Instruments). A 50-micro liter sample of the plasma was pipetted into a scintillation vial, 0.6 ml tissue solubilizer was added, and the vial was capped and then heated to 50 degrees Celsius for 50 min. The sample was neutralized with 15 micro liter glacial acetic acid, and 16 ml scintillation cocktail was added to the sample. All samples were protected from light for 2 days (to reduce chemiluminescence) before counting (1900TR Liquid Scintillation Analyzer. Packard Instruments, Downers Grove, IL) with automatic quench correction. CMR sub G was then calculated using the method of Sokoloff et al., [17] as described by Mujsce et al. [18] A lumped constant of 0.483 was used. The details of other studies related to these $CMR_G$ calculations are described in the appendix.

## Part II: Cerebral Metabolic Rate for Glucose and Depolarization Times

The relations between temperature and depolarization times and between temperature and $CMR_G$ were fitted using a second-order polynomial. By combining these two equations, it was possible to construct a single curve to examine the relation between depolarization times and $CMR_G$ (see Results). (This combination was done empirically; a fixed series of temperature values [at 0.5 degree Celsius intervals] was entered into the equations of temperature versus $CMR_G$ and temperature versus depolarization times, thereby generating an array of paired depolarization time and $CMR_G$ values that we used to draw a third $CMR_G$ versus depolarization curve. This combined curve was fitted with a new second-order polynomial.) However, to compare more directly the effects of hypothermic and anesthetic depression of $CMR_G$, additional experiments were needed. To simplify these studies, CMR sub G and terminal depolarization times were measured in the same animal. Note however, that all blood samples needed for $CMR_G$ calculation were obtained before KCl injection.

All animals were allowed free access to food and water until the time of study. Rats were prepared in a fashion identical to that noted above (bilateral vascular catheters, craniectomy, cortical microelectrode). After surgical preparation was finished, animals were assigned to one of two experimental groups. Note that these groups were chosen on the basis of pilot

studies demonstrating that these conditions resulted in similar $CMR_G$ values.

- Hypothermia (n = 3). Anesthesia was maintained with 0.8% halothane in 50% $N_2$ O/$O_2$. Pericranial and rectal temperature were adjusted to and maintained at approximately 27 degrees Celsius.

- Pentobarbital (n = 3). Anesthesia was maintained with 0.8% halothane/50% $N_2$ O/$O_2$. When preparation was complete, an intravenous infusion of pentobarbital was started at the rate of 5 mg *symbol* kg sup -1 *symbol* min sup -1 kg sup -1 min *symbol*. This was continued until a burst-suppression or isoelectric EEG pattern was obtained. The pentobarbital infusion was`` thereafter reduced to 0.5- 1.5 mg *symbol* kg sup -1 *symbol* kg sup -1 *symbol* min sup -1, adjusted to maintain a stable EEG pattern. Pericranial temperature was maintained at 38 degrees Celsius, and MAP was kept greater than 80 mmHg by infusion of 6% hetastarch or rat donor blood if necessary.

After a stabilization period of 30-50 min, [sup 14 Carbon]2- DG was infused intravenously, and arterial blood sampling was performed as above. At the end of the 45-min sampling period, the animals were killed with 0.5 ml saturated KCl given intravenously, and the time to terminal depolarization was recorded. After depolarization had occurred, the animals were decapitated, and the brains were removed. The isotope contents of plasma and the brain sample were measured and $CMR_G$ were calculated as above.

## Part III: Glucose Effects

On examination of data in part I, it was noted that plasma glucose concentrations increased as temperature decreased. As a result, in part II above, the plasma glucose concentration was significantly greater in 27 degrees Celsius animals than in animals anesthetized with pentobarbital. Because depolarization times can be influenced by plasma glucose, [19,20] we repeated the part II study in animals that had been fasted overnight (with free access to water). In addition to the same two groups (hypothermia to [nearly equal] 27 degrees Celsius [n = 5], pentobarbital [n = 6]), we added a group of animals anesthetized with high-dose isoflurane. Preparation of these animals was as follows:

- Isoflurane (n = 5). After surgical preparation was finished, halothane was discontinued, and anesthesia was maintained with 2.3-2.4% isoflurane in 50% $N_2$ O/$O_2$, adjusted to maintain EEG isoelectricity. Pericranial temperature was maintained at 38 degrees Celsius, and MAP was kept greater than 80 mmHg by infusion of 6% hetastarch or rat door blood if necessary. $CMR_G$ and terminal depolarization times were measured as above.

## Statistical Analysis

Scattergrams of temperature versus depolarization times and of temperature versus $CMR_G$ (part I) were fitted using a second-order polynomial. In addition, the effects of temperature on physiologic parameters (including MAP and blood gases) were examined using polynomial regressions. Intergroup comparisons of $CMR_G$ and depolarization times in parts II and III (hypothermia vs. pentobarbital vs. isoflurane) were examined using a one-way analysis of variance with post hoc Scheffe's test. The ratio of metabolic rates over a temperature interval of 10 degrees Celsius ($Q_{10}$) for the effects of temperature on depolarization times and $CMR_G$ (using a temperature range of 39- 25 degrees Celsius) was calculated as the antilog of the following value:

Equation 1.

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$$\left(\frac{\log\left[\frac{\text{high value}}{\text{low value}}\right]}{\Delta\text{temperature}}\right) \times 10$$

Equation 1

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## Results

### Part I: Hypothermia, Cerebral Metabolic Rate for Glucose, and Depolarization Times

Physiologic Parameters. Values for MAP, arterial blood gases, and hematocrit at three selected temperatures over our study range (39, 32, and 25 degrees Celsius) are summarized in Table 1. These summary data were taken from 12 animals (n = 6 from the depolarization and CMR sub G studies). An examination of the scattergrams constructed using all animals showed no significant relations between temperature and MAP, pH, arterial carbon dioxide tension, and hematocrit. Arterial oxygen tension and plasma glucose values tended to increase with progressive cooling.

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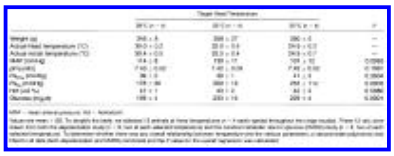

Table 1. Physiologic Parameters versus Temperature

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Terminal Depolarization. The times to terminal depolarization versus measured head temperature are shown in Figure 1(A) (n = 19 animals). Depolarization time increased from a fitted value of 76 s at 39 degrees Celsius to 326 s at 25 degrees Celsius. The calculated $Q_{10}$ was 2.83.

Measurement of Cerebral Metabolic Rate for Glucose. $CMR_G$ values over the tested temperature range (and a fitted regression line) are shown in Figure 1(B) (n = 11 animals). At 39 degrees Celsius. the fitted value of $CMR_G$ was 66 micro Meter *symbol* 100 $g^1$ *symbol* $min^1$, decreasing to 21 micro Meter *symbol* 100 $g^1$ *symbol* $min^1$ at 25 degrees Celsius. The calculated $Q_{10}$ was 2.30.

Cerebral Metabolic Rate for Glucose versus Depolarization Times. By combining two fitted equations for temperature versus depolarization times and $CMR_G$, it was possible to construct a composite graph of $CMR_G$ versus time to depolarization. This is shown as the thin solid line in Figure 2.

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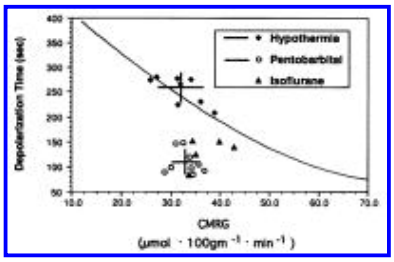


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Figure 2. Data from parts II and III. The continuous curved line depicts the relation between cerebral metabolic rate for glucose (CMR sub G) and depolarization times as derived from the hypothermic data described in part I and was constructed by combining the polynomial equations that were fitted to the scattergrams in figures 1A and 1B. Filled diamonds - data points for the hypothermic animals ([nearly equal] 27 degrees Celsius) in parts II and III, with superimposed x and y standard deviations; open circles = data points for pentobarbital- anesthetized, normothermic rats; dashed crosses = x and y standard deviations; filled triangles - isoflurane animals (SD bars not shown for clarity).

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Parts II and III: Cerebral Metabolic Rate for Glucose and Depolarization Times

$CMR_G$, depolarization times, and plasma glucose concentration for nonfasted (part II) and fasted (part III) animals are shown in Table 2. Although plasma glucose values were significantly greater in nonfasted hypothermic rats than in nonfasted pentobarbital animals (and in nonfasted vs. fasted animals), there were no evident effects of plasma glucose on $CMR_G$ or depolarization times. Therefore, the two groups were combined. $CMR_G$ values in the three groups were similar: hypothermia 32 plus/minus 4, pentobarbital 33 plus/minus 3, and isoflurane 37 plus/minus micro Meter *symbol* 100 g sup 1 *symbol* $min^1$ (P = 0.059), analysis of variance). However, despite these similarities, times to terminal depolarization were significantly different: hypothermia 253 plus/minus 29, pentobarbital 109 plus/minus 24, and isoflurane 130 plus/minus 28 s (P < 0.0001). These data are shown superimposed on the hypothermic $CMR_G$ versus depolarization time line in Figure 2.

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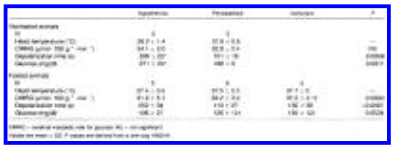

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Table 2. CMRG and Depolarization Times: Hypothermia versus Pentobarbital and Isoflurane

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Electroencephalograms. Data relating postarrest times to EEG isoelectricity versus temperature were examined in halothane/nitrous oxide animals from all groups. A similar analysis was not possible in pentobarbital and isoflurane animals because the EEGs in these animals were markedly suppressed before the onset of ischemia. As with depolarization times, there was a strong inverse relation (P < 0.0005), with a fitted value of 12 s at 39 degrees Celsius and 22 s at 25 degrees Celsius. The calculated $Q_{10}$ value was 1.04.

## Discussion

There is now a large body of information demonstrating that mild hypothermia (30-35 degrees Celsius) provides a remarkable degree of cerebral protection during hypoxia or ischemia, as evidenced by biochemical changes, histopathologic findings, and functional outcome. [1,2,4,5,21-28] Although relatively few studies have directly compared the effect of hypothermia with that of anesthesia, available data suggest that hypothermia is more efficacious. [2,16,29-31] This possibility appears to hold true even when measured or predicted reductions in cerebral metabolic rate are less during hypothermia than during normothermic anesthetic treatment. [2,16].

The current study was undertaken to investigate this apparent inconsistency. To provide a point of reference, we first examined the relations among temperature, $CMR_G$, and the time to terminal depolarization. As expected, we found a strong inverse relation between temperature and the time to depolarization and between $CMR_G$ and the time to depolarization, an observation consistent with metabolic depression as the primary determinant of the time to postischemic membrane failure. [9] With these data it was possible to select a temperature ([nearly equal] 27 degrees Celsius) that resulted in a CMR sub G approximately equal to the that produced by deep pentobarbital and isoflurane anesthesia. A second series of experiments was then carried out to compare $CMR_G$ and depolarization times directly in these three groups. The results indicated that despite similar $CMR_G$ values, hypothermia resulted in substantially longer times to depolarization than did either of the two anesthetics. For example, at $CMR_O$ values of 32-34 micro Meter *symbol* 100 $g^1$ *symbol* min sup 1, the depolarization time with hypothermia was almost 150 s longer than with pentobarbital. This result is very similar to that obtained by Astrup et al. [9] In that study, when pentobarbital was given to rats in a dose sufficient to produce a 35% decrease in $CMR_{O2}$, the time to a depolarization increased from 116 to 170 s. In contrast, when a 45% reduction in $CMR_{O2}$ was produced by hypothermia (27 degrees Celsius), depolarization time was 232 s. However, these authors did not examine a wider range of temperature effects, and hence it was not possible to conclude that there was an effect on depolarization times that was independent of $CMR_{O2}$.

There are several possible explanations for this observation. Hypothermia and anesthetics may have different effects on the rate of ATP decrease after ischemia (despite similar $CMR_G$ s). This possibility is not unreasonable given the primary effect of anesthetics on synaptic activity compared with the more "global" effects of hypothermia and is compatible with the ideas expressed by Michenfelder. [32] Similarly, Michenfelder and Theye [33] showed that when hypothermic or barbiturate-anesthetized dogs with equivalent $CMR_O$ sub 2 s were killed, the rate of ATP depletion was slower with hypothermia. However, they did not examine any "functional" end points, such as depolarization. It is thus possible that membrane depolarization occurs at different ATP concentrations during the two conditions. During normothermia, depolarization occurs when ATP concentrations have decreased to values approximately 30% of normal. [20] The concentrations present at the time of depolarization during hypothermia have not been directly measured, although if the data by Thorn et al. [34] and by Michenfelder and Theye [33] concerning the rate of ATP decrease are examined and combined with our data on depolarization times, the (untested) possibility is raised that the concentration of ATP at the predicted time of depolarization may actually be greater than during normothermia.

It also is possible that hypothermia has direct effects on transmembrane ionic movement that may retard depolarization until lesser ATP concentrations are reached. For example, the hydrolysis of ATP is a major source of Hydrogen sup + during ischemia. [35] Acidosis can directly increase Potassium sup + conductance in heart, thereby increasing the rate of Potassium sup + efflux and perhaps reducing the time to depolarization. [36] If the rate of ATP hydrolysis was slowed by hypothermia, this mechanism could have contributed to the differences in depolarization times. There is no firm evidence to support this possibility, although Astrup et al. did show that the sodium-channel blocking agent lidocaine is capable of slowing the rate of ischemic Potassium sup + loss more than is a barbiturate given to an approximately equal metabolic end point. [16,37] Because depolarization occurs only when the transmembrane potential reaches a threshold value, a direct effect of hypothermia on the rate of Potassium sup + leakage and hence the time needed to reach this threshold voltage is plausible. Of course,

many other mechanisms are possible (and testable). Nevertheless, our results confirm the idea that hypothermia has an effect on postischemic events beyond its effect on cerebral metabolic rate.

It is important to discuss the limitations of the methods used in these experiments. We did not perform an outcome study but chose to determine the time to terminal depolarization as an index of the rate of ischemic membrane failure. After the onset of ischemia, the concentrations of high-energy phosphates (ATP and phosphocreatine) decrease rapidly. As these are depleted, the unchecked leak of Potassium sup + progressively reduces the transmembrane voltage, until the threshold value is reached. Accelerated depolarization then occurs, evidenced by a sudden increase in extracellular Potassium sup +, a decrease in extracellular Calcium$^{2}$+, and a sudden increase in extracellular voltage (as measured relative to an extracranial reference). [11,16,38,39] This depolarization triggers the release of multiple excitatory and inhibitory neurotransmitters, and the resultant Calcium$^{2}$+ influx (by voltage-dependent and agonist-operated channels) sets in motion a cascade of intracellular events that eventually result in cell death. [13,14] In addition, the time to depolarization is strongly influenced by preischemic metabolic rate. [9] As a result of these factors--and because it is an easily detected event--we chose the time to depolarization as our principal indicator of severe neuronal ischemia.

To reach our conclusions, we combined data for animals with varying blood glucose values. Studies have shown that preischemic blood glucose can influence the times to terminal depolarization. [19,20] Nonfasted hypothermic rats had higher glucose values than non-fasted normothermic animals given pentobarbital or isoflurane. For this reason, we examined the influence of glucose by comparing fasted with nonfasted animals and found that variations in plasma glucose between 136 and 271 mg/dl did not influence depolarization times. At first, this result appears to differ from that of earlier studies. However, those experiments demonstrated that changing the glucose concentration from 2.5 mM (45 mg/dl) to 20 mM (360 mg/dl) increased depolarization times by about 60 s. Therefore the variations in glucose concentrations in our animals may have been too small to produce an important effect, and we believe that the differences seen in our study cannot be explained on the basis of varying glucose. In addition, similar differences in depolarization times were seen between fasted hypothermic and barbiturate-anaesthetized animals in which glucose values were similar.

Finally, we used the DG method to assess $CMR_G$ in animals before circulatory arrest. [17] This method measures the amount of deoxyglucose-6-phosphate (DG-6-P) in brain approximately 45 min after the injection of radiolabeled DG. Unlike glucose, DG-6-P cannot continue along the glycolytic pathway and does not diffuse out of the cell; it is effectively trapped in the tissue. Although this method has been used extensively to measure metabolic rate in normothermic conditions, relatively few studies have been performed during hypothermia. [40,41] However, the method requires that some assumptions be made--assumptions that may be incorrect during hypothermia. For example, the method assumes that all of the radioactivity in tissue is in the phosphorylated form--that is, all of the radioactivity is attributable to [sup 14 Carbon]DG-6-P. To ensure complete phosphorylation and to allow for the clearance of unphosphorylated DG from the tissue, a 45- min waiting period is typically used after DG injection. However, Palmer et al. have suggested that in newborn dogs cooled to 20 degrees Celsius, only a small fraction of the tissue radioactivity has been converted to DG-6-P within the 45-min period. [41] If this is true for our animals, measured $CMR_G$ values would overestimate true $CMR_G$, and the error would increase as temperature decreased. We therefore performed additional experiments (see Appendix) to measure directly the influence of temperature on the rate

of phosphorylation. [18,41] Unlike Palmer et al., [41] we found no change in phosphorylation during hypothermia. Whether this discrepancy is attributable to a species or age difference is unknown. There is additional indirect evidence for the accuracy of our $CMR_G$ measurements. For example, the calculated $Q_{10}$ values for the effects of temperature on $CMR_G$ are similar to those calculated by others (for $CMR_G$ and $CMR_O$ sub 2) using Kety-Schmidt methods. [37,42-44] We hence believe, as did McCulloch et al., [40] that no special changes in Sokoloff et al.'s method [17] are needed in hypothermic rats.

In summary, we found that when measured $CMR_G$ was equivalently reduced with hypothermia or with burst-suppression doses of barbiturates and isoflurane, the resultant times to postischemic cortical depolarization differed, with hypothermia producing a significantly greater prolongation. The mechanism of this difference is unknown but reinforces the belief that cerebral protection by anesthetics (and perhaps by hypothermia) involve factors other than simple metabolic suppression.

## Appendix: The Determination of CMR sub G

The equation of Sokoloff et al. for the calculation of CMR sub G [17,45] assumes that all of the radioactivity in sampled brain tissue is that of [sup 14 Carbon]2-deoxyglucose-6-phosphate ([sup 14 Carbon]2-DG-6-P)--that is, that all of the labeled DG in brain has been phosphorylated. In typical studies, this assumption is met by waiting a minimum of 45 min to permit the clearance of unphosphorylated DG from the tissues. However, in 1989, Palmer et al., studying newborn puppies, concluded that at 20 degrees Celsius, less than 20% of the radioactivity in the brain 45 min after injection was that of phosphorylated DG. [41] If this were true in our hypothermic animals, the calculated $CMR_G$ values would be far lower than reported here, and the $Q_{10}$ values would be much larger than calculated.

To examine this question directly, the effects of temperature on the relative proportions of [sup 14 Carbon]2-DG and [sup 14 Carbon]2- DG-6-P were examined. The experimental method was described by Mujsce et al. [18] Rats were allowed free access to food and water until anesthetized. They were then prepared for the measurement of $CMR_G$ as described in Materials and Methods, with pericranial temperature varied between 39 and 25 degrees Celsius. [sup 14 Carbon]2-DG was given as noted. Immediately after the collection of the 45-min blood sample, the animals were decapitated, and the brains removed and immediately frozen in liquid nitrogen. The brain was then dissected on an ice- cooled plate, and tissue samples were obtained from the cortical gray matter. The samples were homogenized with 1 ml of 0 degree Celsius 0.4 N perchloric acid. The homogenate was centrifuged at 5,000 x g for 10 min (still cold). An aliquot of the supernatant fluid was then neutralized with a calculated volume of 4.0 N KOH. The precipitated $KHClO_4$ was removed by centrifugation and the supernatant stored at -20 degrees Celsius until analyzed.

To separate DG from DG-6-P, 0.5 ml tissue extract was passed over an anion-exchange column (bed volume 2 ml, formate form, AG 1-X8 Poly-prep Column, Biorad Laboratories, Hercules, CA). The column was then washed twice with 4-ml aliquots of distilled water. The eluate contained only neutral compounds, specifically [sup 14 Carbon]2-DG and glucose. The column was then washed twice with 4 ml 1 N NaCl to remove any bound [sup 14 Carbon]2-DG-6-P. The [sup 14 Carbon] activity in both eluates was measured by scintillation counting. Total [sup 14 Carbon] activity was the sum of both samples ([sup 14 Carbon]2-DG + [sup 14 Carbon]2-DG-6-P), and the ratio of [sup 14 Carbon]2-DG-6-P to total [sup 14 Carbon] was calculated. When this ratio was plotted against temperature, a regression line was obtained with a slope that did not differ significantly from zero.

To examine this question in an alternative manner, $CMR_G$ was assessed in four hypothermic rats (25 degrees Celsius) that were killed 90 min after DG injection. This interval should provide a longer period for the clearance of unphosphorylated compound. If phosphorylation were incomplete after the normal 45-min waiting period, radioactivity in the brain should decrease during the additional 45 min and the resultant $CMR_G$ values should decrease. However, calculated $CMR_G$ in these 90-min animals did not differ from that calculated after a 45-min waiting period.

From these studies, we conclude that no changes in the usual assumptions used in Sokoloff et al.'s method are needed in hypothermic rats.

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