Conditions for Pharmacologic Evaluation in the Gerbil Model of Forebrain Ischemia

Guy L. Clifton, MD, William C. Taft, PhD, Robert E. Blair, Sung C. Choi, PhD, and Robert J. DeLorenzo, MD, PhD, MPH

We looked at Fio₂, choice of anesthetic, nutritional status, and body temperature in a gerbil model of forebrain ischemia to determine their effect on data interpretation, ischemic outcome, and extent of pharmacologic protection. We subjected 484 gerbils to 5 minutes of forebrain ischemia under different experimental conditions. The gerbils were anesthetized with 3% halothane and inspired 21% O₂, 37% O₂ and 60% N₂O, or 97% O₂. Six groups of gerbils pretreated with 200 mg/kg phenytoin or 2 ml/kg polyethylene glycol (vehicle) underwent ischemia in the fasted or fed state. Three groups of gerbils receiving no pretreatment underwent ischemia with rectal temperatures of 32–33°C, 34–35°C, or 37°C. We counted intact neurons in the CA1 hippocampal sector in brains fixed on Day 7 after ischemia, t tests of square-root-transformed cell counts were used to assess the effect of hypothermia, and analysis of variance of the transformed data was used to test for the effects of phenytoin, Fio₂, and nutritional status. Phenytoin pretreatment provided significant protection from CA1 neuron loss in all groups tested (p<0.001), but the degree of protection varied from 20% to 44%. In spite of significantly higher serum glucose concentrations in fed than in fasted gerbils (173 and 118 mg/dl, respectively), we found no significant effect of nutritional status upon neuron loss in phenytoin- or vehicle-pretreated gerbils. An Fio₂ of 21% significantly decreased the number of viable neurons in both vehicle- and phenytoin-pretreated groups (p<0.03), despite the lack of an effect of hypoxemia on arterial blood gases. Body temperature during ischemia had a dramatic impact on ischemia-induced cell death. Even 2°C of hypothermia provided 100% protection from cerebral ischemia (p<0.0001). We conclude that a minimum of 20 gerbils per group together with rigorous attention to detail are necessary to determine protective effect and therapeutic efficacy with reliance in this widely used model. (Stroke 1989;20:1545–1552)

The gerbil model of forebrain ischemia due to brief bilateral carotid artery occlusion has been widely used to assess pharmacologic protection from cerebral damage. The model’s advantages are a low mortality rate, simplicity of preparation, little morbidity, and a high incidence of cerebral ischemia. The CA1 hippocampal sector shows severe neuronal loss in 85–95% of gerbils by 7 days after occlusion. Although this model can be useful in investigating the pathophysiology of cerebral ischemia, careful attention to experimental parameters may be required to achieve adequate reliability. To investigate the effect of experimental parameters on ischemia-induced cell death, we systematically varied inspired oxygen concentration (Fio₂), anesthetic choice, nutritional status, and body temperature and examined CA1 hippocampal neuron loss in the gerbil model of forebrain ischemia. In addition, we studied the effect of the first three variables on the degree of protection from cell death provided by phenytoin.

Materials and Methods

Groups of 20–40 Mongolian gerbils (Meriones unguiculatus) each underwent 5 minutes of forebrain ischemia under 3% halothane anesthesia with variations in Fio₂ (21% [normoxia], 37%, or 97%), N₂O (0% or 60%), rectal temperature (32–33°C, 34–35°C, or 37°C [normothermia]), and nutritional status (fasted [allowed access to only water for 24 hours before surgery] or fed [allowed ad libitum access to food and water]). Six groups of 40 gerbils each were treated with 200 mg/kg phenytoin 2 hours before ischemia, and six groups of 25 gerbils each were treated with 2 ml/kg polyethylene glycol (vehicle). The six experimental conditions to which

From the Department of Surgery, Division of Neurosurgery (G.L.C.), the Department of Neurology (W.C.T., R.E.B., R.I.D.), and the Department of Biostatistics (S.C.C.), Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Address for reprints: Guy L. Clifton, MD, Medical College of Virginia, Box 677, MCV Station, Richmond, VA 23298-0677.

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a group could be subjected were: 1) FIO₂ of 21%, 0% N₂O, normothermia, fasted; 2) FIO₂ of 21%, 0% N₂O, normothermia, fed; 3) FIO₂ of 37%, 60% N₂O, normothermia, fasted; 4) FIO₂ of 37%, 60% N₂O, normothermia, fed; 5) FIO₂ of 97%, 0% N₂O, normothermia, fasted; or 6) FIO₂ of 97%, 0% N₂O, normothermia, fed. Three groups of 20 gerbils each, pretreated with neither phenytoin nor vehicle, underwent ischemia at an FIO₂ of 37%, 60% N₂O, and rectal temperatures of 32–33°C, 34–35°C, or 37°C.

Other than these differences in experimental conditions, management of the gerbils during induction of anesthesia, during surgery, and during the postoperative period was highly standardized. The gerbils were anesthetized in an insulated chamber with a controlled temperature, which varied with the experimental conditions. Anesthesia induction required 4 minutes and was always with 3% halothane; FIO₂ and the presence of N₂O varied with the experimental conditions. After induction of anesthesia, the gerbils were transferred to an operating table either warmed (for normothermia), at room temperature (for 34–35°C), or slightly cooled (for 32–33°C). Subsequent surgery, ischemia, and wound closure required 15 minutes.

A ventral cervical incision exposed both common carotid arteries, which were occluded for 5 minutes using Heifitz aneurysm clips. Occlusion and restoration of blood flow after clip removal was confirmed visually using 3.5× magnification. During the first 4 minutes of ischemia, the gerbils received no halothane but received O₂ and/or N₂O as dictated by the experimental conditions. Halothane was reinstituted 1 minute before clip removal to provide anesthesia during wound closure. The indicated gas was administered for 20 minutes; for 10 minutes before ischemia, for the 5 minutes of ischemia, and for 5 minutes after ischemia. Rectal temperature was monitored continuously during surgery, ischemia, and wound closure. After wound closure, the gerbils were kept in a constant environmental temperature of 30°C for 7 days. Rectal temperatures were not monitored after wound closure.

Femoral arterial blood gases were measured in 27 untreated normothermic fed gerbils 5 minutes after the onset of ischemia under 3% halothane anesthesia with an FIO₂ of 97% (n=8), 37% (and 60% N₂O) (n=8), or 21% (n=11). Femoral arterial blood gases were also measured in eight untreated normothermic fed gerbils that underwent sham operation while inspiring 21% O₂ under 3% halothane anesthesia. Femoral arterial serum glucose concentration was measured in 35 normothermic fasted gerbils and in 46 normothermic fed gerbils 5 minutes after the onset of ischemia under 3% halothane anesthesia with an FIO₂ of 21% (n=18 and 19, respectively), 37% (and 60% N₂O) (n=11 and 19, respectively), or 97% (n=6 and 8, respectively). Femoral arterial serum glucose concentration was also measured in eight normothermic untreated fasted and 14 normothermic untreated fed gerbils that underwent sham operation. After 7 days, the gerbils were killed, perfused, and their brains were processed for histologic examination as described. The brains were sectioned coronally and dehydrated in a series of ethanol (60–100%) and xylene baths before being embedded in paraffin; 7-µm slices containing the dorsal hippocampus were cut with an American Optical AL microtome (Buffalo, New York) and slide-mounted before staining with cresyl violet. The stained sections were then examined using light microscopy. The number of viable neurons in the CA1 sector of the hippocampus was counted using an American Optical Microscope at 400×. Cells in the 3-mm-long CA1 sector were counted on both the right and left sides, and the values were averaged to derive the cell count per millimeter for each gerbil. The cell counts were categorized as: no protection (0–40 cells/mm), moderate protection (41–199 cells/mm), and substantial protection (>200 cells/mm) from neuronal loss. Ten sham-operated gerbils and 10 unoperated gerbils underwent the same histologic procedures, and neurons in the CA1 sector were counted.

The square roots of the cell counts were taken to normalize the data. The global effects of phenytoin, FIO₂, nutritional status, and their interactions were studied using analysis of variance (ANOVA) of the transformed data. ANOVA was augmented by the two-sample t test to compare two corresponding groups. Minimum sample size to detect a significant (p<0.05) effect was determined from the mean and standard deviation of transformed cell counts of phenytoin- and vehicle-pretreated groups with an FIO₂ of 37% or 97% using t tests. Percent neuronal protection for each condition was calculated as the ratio of intact neurons per millimeter of CA1 length in phenytoin-pretreated gerbils to the difference between cell counts in sham-operated and vehicle-pretreated gerbils in the same experimental condition.

### Results

The mean number of viable neurons in the CA1 sector of the hippocampus in the 10 sham-operated gerbils was 254/mm. Five minutes of bilateral carotid artery occlusion in gerbils, which produces global cerebral ischemia, causes dramatic histologic death of hippocampal CA1 pyramidal neurons (Figure 1, C and D). Although phenytoin pretreatment provided significant neuronal protection under all experimental conditions, the degree of protection varied from 19.7% to 44.2% (Table 1). This wide range illustrates the necessity of rigorous attention to experimental parameters, both in investigating the mechanisms that underlie the pathophysiology of ischemia and in therapeutic testing.

Among vehicle-pretreated gerbils, all groups showed significant ischemia-induced cell death, but slightly larger numbers of viable neurons were seen...
in the fed group with an Fio$_2$ of 97%. Among phenytoin-pretreated gerbils, the lowest cell counts and the lowest degrees of neuronal protection were observed in the groups with an Fio$_2$ of 21%; no substantial differences in cell count or neuronal protection were noted among the groups with Fio$_2$s of 37% (and 60% N$_2$O) and 97%. ANOVA demonstrated that decreasing Fio$_2$ to 21% significantly diminished neuronal protection by phenytoin (p<0.03). By t test, significantly lower cell counts were found in both fasted and fed groups with an Fio$_2$ of 21% than in groups with greater Fio$_2$s.
TABLE 1. Viable Neurons/mm in CA1 Hippocampal Sector of Gerbils Pretreated With Phenytoin or Vehicle Under Various Conditions During 5 Minutes' Bilateral Common Carotid Artery Occlusion

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fio2</td>
<td>N2O</td>
</tr>
<tr>
<td>21% 0%</td>
<td>Fasted</td>
</tr>
<tr>
<td>21% 0%</td>
<td>Fed</td>
</tr>
<tr>
<td>37% 60%</td>
<td>Fasted</td>
</tr>
<tr>
<td>37% 60%</td>
<td>Fed</td>
</tr>
<tr>
<td>97% 0%</td>
<td>Fasted</td>
</tr>
<tr>
<td>97% 0%</td>
<td>Fed</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Overall effect of phenytoin is significant at p<0.0001 by ANOVA.

N2O at 60% was administered to the group with an Fio2 of 37%. Since this combination of gases is commonly used for anesthesia, a detrimental effect of N2O compared with pure O2 (Fio2 of 97%) or room air (Fio2 of 21%) was investigated, but none was found. It cannot be excluded that some beneficial effect of 37% O2 compared with 97% O2 was offset by a detrimental effect of N2O, though this seems unlikely.

No significant differences in pH or Paco2 were seen among the groups that underwent ischemia at Fio2s of 21%, 37%, or 97% (Table 2). However, the groups with Fio2s of 37% and 97% showed the expected increase in Pao2 from the value in the sham-operated group. Hypocapnia and increased pH, reflecting moderate hyperventilation, was seen in all three ischemic groups compared with the sham-operated group. Hypoxia (Pao2 of <70 mm Hg) was not found in any group or in any gerbil.

Among vehicle-pretreated gerbils, cell counts were lower in fasted than in fed groups with Fio2s of 21% and 97%, but not 37% (Table 1). Among phenytoin-pretreated gerbils, cell counts were higher in fasted than in fed groups with Fio2s of 21% and 37%; cell counts were lower in the fasted group with an Fio2 of 97%. The highest cell counts were noted in the phenytoin-pretreated group with an Fio2 of 37% and in the phenytoin-pretreated fed group with an Fio2 of 97%.

TABLE 2. Arterial Blood Gases in Gerbils Under Various Conditions During 5 Minutes' Bilateral Common Carotid Artery Occlusion

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Blood gases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td>Fio2</td>
</tr>
<tr>
<td>Yes 21% 0%</td>
<td>Fed</td>
</tr>
<tr>
<td>Yes 37% 60%</td>
<td>Fed</td>
</tr>
<tr>
<td>Yes 97% 0%</td>
<td>Fed</td>
</tr>
<tr>
<td>No* 21% 0%</td>
<td>Fed</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

*Sham operation.

TABLE 3. Blood Glucose Concentration in Gerbils Under Various Conditions During 5 Minutes' Bilateral Common Carotid Artery Occlusion

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Fasted</th>
<th>Nutritional status</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td>Fio2</td>
<td>N2O</td>
<td>n</td>
</tr>
<tr>
<td>Yes 21% 0%</td>
<td>18</td>
<td>119±7</td>
<td>19</td>
</tr>
<tr>
<td>Yes 37% 60%</td>
<td>11</td>
<td>116±7</td>
<td>19</td>
</tr>
<tr>
<td>Yes 97% 0%</td>
<td>6</td>
<td>116±9</td>
<td>8</td>
</tr>
<tr>
<td>Average</td>
<td>35</td>
<td>118±4</td>
<td>46</td>
</tr>
<tr>
<td>No* 21% 0%</td>
<td>8</td>
<td>114±6</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are mean±SEM mg/dl. Under all conditions, fasted blood glucose concentrations are significantly different from fed concentrations at p<0.0005 by t test.

*Sham operation.
gerbils. ANOVA showed no significant effect of nutritional status.

Blood glucose studies predictably demonstrated that fasted gerbils had consistently lower plasma glucose levels than fed gerbils (Table 3). No significant differences were found between ischemic and sham-operated fasted or fed groups, and Fio$_2$ did not affect plasma glucose concentration.

A small (2–3°C) drop in rectal temperature produced near-complete histologic protection from ischemia-induced CA1 neuron loss; complete protection was observed with a greater (4–5°C) degree of hypothermia (Table 4). Cell counts in both hypothermic groups did not differ significantly from those of sham-operated or unoperated groups. The protection achieved by even mild hypothermia was significantly greater than that in the most highly protected group of normothermic phenytoin-pretreated gerbils (see below).

The degree of neuronal protection achieved by pretreatment with phenytoin was not consistent among gerbils, but ranged from no protection to substantial protection (Table 5). In the vehicle-pretreated groups, almost all gerbils (76–100%) fell in the “no protection” category and few gerbils (0–5%) fell in the “substantial protection” category. Phenytoin pretreatment shifted the distribution of neuronal protection; 25–44% of the gerbils exhibited moderate protection and 8–27% exhibited substantial protection.

The “moderate protection” category compromised many gerbils with substantial protection in one hemisphere and little protection in the other, resulting in asymmetry. Asymmetry was rarely observed in vehicle-pretreated gerbils, but 14–26% of the phenytoin-pretreated gerbils showed obvious asymmetry (Table 5). Thus, only 11–18% of the phenytoin-pretreated gerbils showed true bilateral neuronal protection of a moderate degree. No significant effect of Fio$_2$ or nutritional status on the asymmetry of phenytoin protection was noted. All hypothermic gerbils fell into the “substantial protection” category (Table 4) and no asymmetry was noted. Based on data from the phenytoin- and vehicle-pretreated groups with Fio$_2$s of 37% and 97%, a minimum of 20 animals per group is required to detect a significant effect of the drug.

**Discussion**

In this gerbil model, we have shown that the use of 60% N$_2$O with 37% O$_2$ does not reduce neuronal protection by phenytoin compared with an Fio$_2$ of 97% (Table 1). Despite the lack of evidence of an effect of hypoxemia on arterial blood gases (Table 2), an Fio$_2$ of 21% significantly reduced the degree of neuronal protection provided by phenytoin compared with an Fio$_2$ of 97% or the combination of 37% O$_2$ and 60% N$_2$O (Table 5). Significant differences in serum glucose concentrations were found between fasted and fed ischemic groups (Table 3).

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**Table 4. Cell Counts at 7 Days in Untreated Gerbils Subjected to 5 Minutes’ Bilateral Common Carotid Artery Occlusion at Different Rectal Temperatures**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Rectal temperature (°C)</th>
<th>Viable neurons/mm (mean±SEM)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32–33</td>
<td>20</td>
<td>257±4</td>
</tr>
<tr>
<td>Yes</td>
<td>34–35</td>
<td>20</td>
<td>250±13</td>
</tr>
<tr>
<td>Yes</td>
<td>37</td>
<td>18</td>
<td>16±8</td>
</tr>
<tr>
<td>No*</td>
<td>37</td>
<td>10</td>
<td>254±4</td>
</tr>
<tr>
<td>No†</td>
<td>37</td>
<td>10</td>
<td>262±3</td>
</tr>
</tbody>
</table>

*Sham operation.
†Unoperated.

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**Table 5. Degree and Asymmetry of Neuronal Protection at 7 Days in Phenytoin- and Vehicle-Pretreated Gerbils Subjected to 5 Minutes’ Bilateral Common Carotid Artery Occlusion Under Different Conditions**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Nutritional status</th>
<th>Fio$_2$</th>
<th>N$_2$O</th>
<th>Viable neurons/mm</th>
<th>Asymmetry</th>
<th>Viable neurons/mm</th>
<th>Asymmetry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>21% 0% Fasted</td>
<td></td>
<td></td>
<td></td>
<td>36</td>
<td>23</td>
<td>64%</td>
<td>9</td>
</tr>
<tr>
<td>21% 0% Fed</td>
<td></td>
<td></td>
<td></td>
<td>38</td>
<td>21</td>
<td>55%</td>
<td>14</td>
</tr>
<tr>
<td>37% 60% Fasted</td>
<td></td>
<td></td>
<td></td>
<td>37</td>
<td>15</td>
<td>41%</td>
<td>12</td>
</tr>
<tr>
<td>37% 60% Fed</td>
<td></td>
<td></td>
<td></td>
<td>39</td>
<td>21</td>
<td>54%</td>
<td>13</td>
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<tr>
<td>97% 0% Fasted</td>
<td></td>
<td></td>
<td></td>
<td>34</td>
<td>15</td>
<td>44%</td>
<td>12</td>
</tr>
<tr>
<td>97% 0% Fed</td>
<td></td>
<td></td>
<td></td>
<td>39</td>
<td>14</td>
<td>36%</td>
<td>17</td>
</tr>
</tbody>
</table>
However, these differences were not associated with changes in the degree of neuronal protection exerted by phenytoin nor were they associated with changes in the lack of neuronal protection exerted by vehicle (Table 5). Mean cell counts in vehicle-pretreated gerbils varied from 2% to 14% (Table 1) of those in sham-operated groups, suggesting stability of neuronal loss in vehicle-pretreated gerbils (data not shown). We show that a decrease in rectal temperature of 2°C from normothermia before and during ischemia results in almost-complete histologic protection of the CA1 hippocampal sector from 5 minutes of cerebral ischemia. Because of these and other variables in this model, a minimum of 20 animals per group is needed to acquire statistically meaningful data.

We examined the effect of FiO2 because the inhalation of hyperoxic gases commonly used during induction of anesthesia may increase PaO2, which in turn may increase tissue O2 concentration, which could increase cellular oxidation reactions. Specifically, lipid peroxidation has been implicated in the pathophysiology of cerebral ischemia. Lipid peroxidation is a result of the action of oxygen free radicals on lipids and can be produced after ischemia in vitro in brain homogenates by exposing them to 100% O2. In the gerbil model of 15 minutes forebrain ischemia, exposure to 100% O2 for 3–6 hours after ischemia increases mortality threefold; an increase in pentane production in gerbils inspirating 100% O2 compared with those inspiriting room air suggested that supplemental oxygen during reperfusion increased lipid peroxidation. In the gerbil model that we employed, inhalation of 97% O2 for 20 minutes did not diminish the neuronal protection provided by phenytoin. Gerbils breathing 21% O2 were not hypoxic (Table 2), as might have resulted from a pulmonary shunt induced by cerebral ischemia, yet cell counts at 7 days in these gerbils were significantly lower than those in gerbils breathing hyperoxic gases (Table 1). Neurogenic pulmonary edema with hypoxia commonly occurs after neuronal injury in rodents and may be exacerbated by anesthetics. Hypoxia from neurogenic pulmonary injury did not occur in these experiments.

The mechanism of the enhanced neuronal protection by hyperoxic gases that we found in these experiments is not known. It is unlikely that the administration of hyperoxic gases (FiO2 of 37% or 97%) significantly diminished the duration of tissue hypoxia after ischemia compared with normoxia. The rate of decrease of hippocampal and cortical PO2 has been measured in the gerbil brain from the moment of carotid artery occlusion to the time tissue PO2 reached 0; over a range of tissue PO2 (20–50 mm Hg), the rate of decrease increased with increasing PO2 so that brain PO2 fell to 0 within 2–3 seconds of carotid artery occlusion. This rapid rate of decrease of tissue PO2 during ischemia makes it unlikely that increased brain PO2 due to the administration of hyperoxic gases significantly diminished the duration of brain hypoxemia.

In restrained, unparalyzed rats, 70–80% N2O has been found to have no global effect on cerebral blood flow (CBF) and glucose utilization. Regionally, however, 70–80% N2O decreased CBF in some regions (not the hippocampus) and increased glucose utilization in the red nucleus and lateral geniculate body. In humans, N2O has been found to have variable effects on CBF and cerebral oxygen utilization (CMRO2), either increasing both parameters or having no net effect. In dogs, N2O has been found to increase both parameters. Halothane, while increasing CBF, may decrease CMRO2 in the presence of N2O. It is possible that N2O increases cerebral metabolic rate in this gerbil model, which might worsen the effect of cerebral ischemia. Sixty percent N2O has been reported to reverse the protective effect of thiopental on mortality due to hypoxia in mice. Despite these concerns regarding the use of N2O, 37% O2/60% N2O resulted in a neuronal protection equivalent to that achieved with 97% O2 alone. The advantages of using N2O in experiments such as these are a more rapid induction of anesthesia than with halothane alone and lower halothane concentrations required for anesthesia. We found no disadvantage to using 60% N2O in addition to halothane.

Glucose infusion resulting in plasma glucose levels of 250–800 mg/dl has been shown to exacerbate the effects of incomplete global ischemia and of hypoxia by a number of investigators using both neurologic and histologic end points. Hyperglycemia has been associated with decreased intracellular pH, increased brain lactic acid accumulation, impaired recovery of adenosine triphosphate (ATP), and delayed recovery of CBF. The extent of hyperglycemia associated with exacerbation of ischemia and hypoxia has been studied in several species. In a rodent model of hypoxia-ischemia, Welsh et al concluded that when serum glucose levels exceeded a threshold of 225 mg/dl, recovery of ATP levels were markedly impaired. Gardiner et al found significantly less brain lactate accumulated after hypoxia in fasted (serum glucose concentration 115 mg/dl) than in fed (serum glucose concentration 174 mg/dl) rats or in fasted, glucose-injected rats (serum glucose concentration 155–271 mg/dl); these investigators suggested that serum glucose values of <115 mg/dl allowed enhanced recovery of brain energy metabolism in the immediate posthypoxic period. In hypoxic cats, serum glucose levels of >250 mg/dl have been associated with worsened histologic damage. Data in humans suggests that serum glucose concentrations of >300 mg/dl adversely affect the neurologic outcome of cardiac arrest. Thus, the preponderance of evidence suggests that serum glucose values exceeding 150–200 mg/dl have a deleterious effect after cerebral ischemia.
We found 47% higher intraschismic serum glucose values in fed than in fasted gerbils (173 and 118 mg/dl, respectively; Table 3), but the variation was not associated with impairment of neuronal protection by phenytoin (Table 1), with increased mortality, or with worsened neuronal loss in vehicle-injected gerbils (Table 5). It is likely that these serum glucose levels are below the threshold for increased lactate accumulation and exacerbation of cerebral ischemia in this model. The only effect of a 48-hour fast upon brain energy substrate utilization is a small decrease in glucose utilization. The potential for stress- or anesthetic-induced hypoglycemia in fed gerbils, however, is greater than that in fasted gerbils. Therefore, the use of fasted animals in experiments of cerebral ischemia offers potential advantages, especially if longer durations of ischemia, and hence more stress, are to be used in some experiments.

The cerebral protective effects of profound hypothermia have long been known, though the mechanism is not understood. Deep hypothermia (8–10°C) is currently used for cerebral protection during the circulatory arrest necessary for correction of some pediatric cardiac anomalies. The cerebral metabolic rate at a core body temperature of 18–19°C is 50% of that at normothermia. Hypothermia to 18–19°C delays depletion of ATP after decapitation, whereas an equivalent reduction of CMRO₂ brought about by anesthetics (providing no ischemic protection) has no effect on depletion of high-energy phosphates after decapitation in dogs. Hence, hypothermic cerebral protection may not be due to the decrease in CMRO₂ as such.

Very mild hypothermia, expected to induce a 5–10% decline in CMRO₂, has only recently been reported to provide cerebral protection from ischemia. Bustos and colleagues have shown that a 2°C reduction in brain temperature during ischemia markedly decreases neuronal damage in the rat model of four-vessel occlusion. In that model, brain temperature fell as much as 6°C below rectal temperature during ischemia unless the rat's head was warmed with a heating lamp. In the gerbil model that we used, disparities between brain and rectal temperatures were minimized and were consistent because the animals were placed in a supine position with the gerbils' bodies and calvariae resting on a heated table. The observation that a 2°C reduction in rectal temperature could provide total histologic protection in the CA1 hippocampal sector in this model was not expected. Since anticonvulsants and anesthetics readily induce hypothermia in animals, heated induction chambers, heated operating tables, and heated recovery chambers have been necessary to prevent mild hypothermia. Without constant measurement of rectal temperature, standard provisions for warming the head, and specially warmed environments, erroneous conclusions regarding pharmacologic protection could easily be reached. Seasonal variations in ambient temperature may also alter results by affecting body temperature.

One interpretation of our finding of either high or low degrees of unilateral cell loss is that of an electrophysiologic threshold phenomenon after ischemia. This hypothesis states that at a certain level of biochemical insult, widespread unilateral neuronal discharge could lead to severe cell loss in that entire CA1 sector. Postischemic neuronal hyperactivity is known to occur in this gerbil model and has been related to selective neuronal vulnerability. Asymmetry of neuronal protection in phenytoin-pretreated gerbils was common. The absence of this finding in ischemic vehicle-pretreated gerbils, however, makes asymmetry of CBF during carotid artery occlusion unlikely.

Based on degrees of neuronal protection found with phenytoin pretreatment, a minimum of 20 vehicle-injected and 20 drug-injected gerbils will be required to reliably detect a significant drug effect. Protective effects using few gerbils have been reported with this model. Given variations in the extent of protection seen with small groups of consecutively injured animals (on a day-to-day basis), conclusions based on small numbers are risky.

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**KEY WORDS** • animal models • cerebral ischemia • phenytoin • gerbils • hypothermia
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