Differential Effects of Anesthetic Agents on Outcome from Near-complete but Not Incomplete Global Ischemia in the Rat

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Background: It has been postulated that anesthetic agents that reduce cerebral metabolic rate will protect the brain against ischemia when electroencephalographic (EEG) activity is persistent, but will provide no protection when ischemia is severe enough to cause EEG isoelectricity. No outcome studies have addressed this issue. The authors studied anesthetic agents to determine if they provide differential effects on outcome from global cerebral ischemic insults that cause either an attenuated or isoelectric EEG.

Methods: Fasted rats were subjected to either (1) incomplete ischemia (attenuated EEG; 20 min of mean arterial pressure [MAP] = 50 mmHg and bilateral carotid occlusion) or (2) near-complete ischemia (isolectric EEG; 10 min of MAP = 50 mmHg and bilateral carotid occlusion) while anesthetized with 1.4% isoflurane, 1 mg·kg⁻¹·min⁻¹ ketamine, or 25 µg·kg⁻¹·h⁻¹ 70% nitrous oxide and fentanyl. The brain was maintained at normothermia during ischemia and for 22 h after ischemia. Five days later, hippocampal CA1 and cortical injury were measured.

Results: There was no difference among anesthetic agents during incomplete ischemia for mean ± SD percentage dead CA1 neurons (fentanyl, 38% ± 20%; isoflurane, 31% ± 10%; ketamine, 40% ± 19%; P = 0.38). During near-complete ischemia, there was a difference among anesthetic agents (fentanyl, 88% ± 9%; isoflurane, 37% ± 20%; ketamine, 70% ± 28%; P = 0.00008). Isoflurane was protective compared with fentanyl (P = 0.00007) and ketamine (P = 0.0061). There was no difference between fentanyl and ketamine (P = 0.145). Similar observations were made in the cortex. Neurologic function correlated with histologic damage.

Conclusions: Outcome from near-complete but not incomplete cerebral ischemia depended on the anesthetic agent administered during the ischemic insult. (Key words: Depolarization; fentanyl; hippocampus; histology; isoflurane; ketamine.)

FOR more than three decades it has been believed that anesthetic agents favorably effect outcome from brain ischemia. It has also been held that the magnitude of anesthetic effect depends on both the severity of the ischemic insult and the choice of anesthetic agent. Focal ischemic insults caused by occlusion of a vessel distal to the circle of Willis have been found to be sensitive to effects of various anesthetic agents. In contrast, some studies have indicated that outcome from severe global ischemia is largely resistant to the beneficial effects of any anesthetic agent.

One factor believed important in explaining why some ischemic insults will be sensitive to anesthetic agents is the magnitude of residual synaptic activity present during the ischemic insult. A seminal experiment performed in dogs revealed that only when the insult allowed persistent EEG activity did the presence of thiopental influence the rate of depletion of high-energy phosphate concentrations. Because anesthetic agents are thought to reduce brain energy requirements
principally by reducing synaptic activity, it can be hypothesized that anesthetic agents will protect the brain only when the ischemic insult allows persistence of some synaptic activity for the anesthetic agent to suppress.

This hypothesis has never been tested directly. We were led to examine this issue for several reasons. Anesthetics that similarly reduce cerebral metabolic rate (CMR) do not uniformly protect the brain from focal ischemic injury. Some drugs that have demonstrable anesthetic effects, despite causing an increase in CMR, reduce ischemic brain damage. Furthermore, anesthetic agents that have markedly different mechanisms of action and markedly different effects on CMR all produce marked neuroprotection in a model of hemispheric global ischemia. Finally, mild hypothermia, which has little effect on the electroencephalogram (EEG) or CMR, has a marked neuroprotective effect when administered either during or after severe global ischemia. These findings indicate that anesthetics have potential mechanisms of action other than depression of CMR during ischemia and that potential neuroprotection of these agents, as a function of EEG state, should be reconsidered.

Accordingly, an incomplete forebrain ischemic insult was used in the rat that allowed persistent EEG activity but resulted in selective neuronal necrosis in the cerebral cortex and hippocampus. Other rats were subjected to a near-complete forebrain ischemic insult that caused both EEG isoelectricity and selective neuronal necrosis. These experiments were designed under these conditions. We hypothesized that differential anesthetic effects on histologic and behavioral outcome would be present in rats subjected to the incomplete but not near-complete global ischemic insult.

Methods

Experiment 1

These studies were approved by the Duke University Animal Care and Use Committee.

Male Sprague-Dawley rats (aged 8–10 weeks; Harlan Sprague-Dawley, Indianapolis, IN) were anesthetized with 50 mg/kg intraperitoneal sodium pentobarbital (Nembutal, 50 mg/ml; Abbott Laboratories, North Chicago, IL). Each animal was positioned in a stereotactic head frame. Using an aseptic technique, the skin was infiltrated with 1% lidocaine and a midline scalp incision was made. A burr hole was drilled over the right hemisphere, 2 mm lateral to the midline and 3 mm anterior to the bregma. A radiotelemetry thermistor (Brain Probe, model XM-FH-BP, Mini-Mitter, Sunriver, OR), accuracy ± 0.1°C, was placed on the skull with the tip positioned on the dura. The probe was fixed in place with two cranial screws and the burr hole was sealed with orthodontic cement. The wound was closed with sutures, and the animals were allowed to awaken.

The thermistor was previously calibrated (within the range of 35–40°C) in a circulating water bath against a mercury thermometer. This allowed extrapolation of temperatures from calibration points in accordance with the radiofrequency emitted by the probe. Radiofrequency signals from the probe were received (Telemetry Receiver model RA1010; Data Science, St. Paul, MN), digitized, and processed through a computer (4DX-33V; Gateway 2000, North Sioux City, SD) with software allowing monitoring and automated control of brain temperature.

After a 2- to 5-day recovery interval, rats were fasted from food for 12–16 h but allowed free access to water. The animals were then anesthetized with 5% isoflurane in oxygen. After orotracheal intubation, the lungs were mechanically ventilated (30% oxygen–balance nitrogen). The inspired isoflurane concentration was reduced to 2% to 2.5%. Surgery was performed with an aseptic technique, and all surgical fields were infiltrated with 1% lidocaine. The tail artery was cannulated and used for blood pressure monitoring and arterial blood sampling. Via a ventral neck incision, the right jugular vein was cannulated with a silicone catheter to infuse drugs and withdraw blood. The common carotid arteries were encircled with sutures. The vagus nerves and cervical sympathetic plexi were left intact. Muscle paralysis was provided by a 1-mg intravenous bolus of succinylcholine, repeated as necessary to allow ventilation control. Pilot studies had been done to ensure that rats would not exhibit an escape response in the absence of succinylcholine given the respective anesthetic regimens. Bilateral cortical EEG activity was monitored continuously during the experiment from active subdural electrodes positioned over the parietal cortex bilaterally, a reference electrode placed on the nasion, and a ground lead positioned in the tail. Heparin (50 IU) was given intravenously.

After surgical preparation, a 20-min interval was allowed for physiological stabilization. Rectal temperature was monitored by a temperature probe (YSI Telethermometers model 401, Yellow Spring Instruments,
Yellow Spring, OH) and servedregulated at 37.5 ± 0.1°C by surface heating and cooling. Brain temperature was controlled at 37.5°C by surface heating and cooling during ischemia and for the first 22 h of recovery.

Rats were randomly assigned to one of three anesthetic regimens. (1) Isoflurane 1.4% was inspired in 30% oxygen–balance nitrogen. (2) Nitrous oxide–fentanyl was given, with isoflurane discontinued. An intravenous infusion of fentanyl was begun (10 μg/kg bolus followed by 25 μg·kg⁻¹·h⁻¹). The inspiratory gas mixture was changed to 30% oxygen and 70% nitrous oxide. (3) Ketamine was given, with isoflurane discontinued. The inspiratory gas mixture was maintained at 30% oxygen–balance nitrogen. Ketamine was infused intravenously at a rate of 1 mg·kg⁻¹·min⁻¹.

Doses of anesthetic agents were chosen based on previous reports that isoflurane and ketamine were neuroprotective at these doses when compared with nitrous oxide or nitrous oxide and fentanyl in a model of hemispheric ischemia. A 30-min interval was allowed to establish the respective anesthetic states. Ventilation was adjusted to maintain a partial pressure of arterial carbon dioxide within 36–42 mmHg.

Mean arterial pressure (MAP) was reduced by exsanguination to either (1) 30 mmHg (isoelectric EEG) or (2) 50 mmHg (attenuated EEG), followed by bilateral carotid occlusion using temporary aneurysm clips. A pilot study determined that a blood pressure of 50 mmHg would reliably produce an attenuated EEG during ischemia with approximately ~40% dead hippocampal CA1 neurons occurring in the fentanyl–nitrous oxide group with few animal deaths. Ischemia persisted for 10 min in the MAP = 30 mmHg group and 20 min in the MAP = 50 mmHg group. To terminate ischemia, shed blood was reinfused and carotid arteries were deoccluded. To minimize systemic acidosis, 0.1 mEq NaHCO₃, was given intravenously.

Anesthetic agents were continued after ischemia for variable periods (isoflurane group, 110 min; fentanyl–nitrous oxide group, 80 min; and ketamine group, 80 min) to ensure that animals in all groups would recover the righting reflex at approximately 2 h after onset of reperfusion. Tracheas were extubated and rats placed in an oxygen-enriched recovery chamber (inspired oxygen fraction, 0.3–0.4) to allow temperature control for an additional 22 h. Rats were returned to their cages with free access to water and food for 5 days.

On the fifth postoperative day, motor function tests were performed. Brieferly, the rats were placed on a 29 × 30 cm screen (grid size, 0.6 × 0.7 cm) that could be rotated from 0° (horizontal) to 90° (vertical). The animal was placed on the horizontal screen and the screen was then rotated into the vertical plane. The duration of time that the animal was able to hold onto the vertical screen was recorded to a maximum of 15 s (allowing a total of three points). Next, the animal was placed at the center of a horizontal wooden rod (2.5 cm diameter) and the time that the animal was able to remain balanced on the rod was recorded to a maximum of 30 s (allowing a total of 3 points). Finally, a prehensile traction test was administered. The time that the animal was able to cling to a horizontal rope was recorded to a maximum of 5 s. From these three tests, a total motor score (9 possible points) was computed.

Rats were anesthetized with halothane and underwent in situ brain fixation by intracardiac injection of buffered 4% formalin. After overnight stabilization, the brains were removed and stored in 4% formalin. Paraffin-embedded brain sections were serially cut (5 μm thick) and stained with aniline blue. Injury to the CA1 sector of the hippocampus was visualized by light microscopic examination. Viable and nonviable neurons were counted manually, and the percentage of nonviable neurons was calculated (% CA1 dead). At the level where the septal nuclei were widest, damage in the neocortex was graded (crude damage index) on a 0–4 scale (0 = no observed histologic change; 1 = 1–5% neurons with pathologic changes; 2 = 6–50% neurons damaged; 3 = 51–100% of neurons damaged; 4 = infarction). By convention, values from the hemisphere with the worst damage were used for the final analysis.

The presence of EEG isoelectric or attenuation was confirmed post hoc by investigators blinded to group assignment. If a rat demonstrated isoelectricity during incomplete ischemia, results from that animal were discarded. If a rat showed EEG activity during near-complete ischemia, data from that animal were discarded.

Statistical analyses of the two primary end points (hippocampal and cortical injury) were chosen a priori. Although concurrently performed, results from the incomplete and near-complete studies were analyzed independently. Within each experiment, hippocampal injury was compared using analysis of variance and Tukey’s method. There was a significant outlier in the near-complete ischemia ketamine group. Because the outlier had the effect of increasing the probability value, the outlier was retained in the analysis. For cortical injury and total motor score, comparisons among

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groups were performed using the Kruskal-Wallis test. Physiologic values were compared among groups by one-way analysis of variance. To validate the total motor score, values for all rats were individually compared with the percentage of dead CA1 neurons and the neocortical CDI using Kendall’s τ (StatXact 3 for Windows, Cytel Software Corp., Cambridge, MA). Statistical significance was assumed when \( P < 0.05 \).

**Experiment 2**

In experiment 1, only surface EEG was recorded. The following study was performed to examine effects of near-complete and incomplete ischemia on EEG and depolarization phenomena in the hippocampus. Animals were anesthetized with isoflurane and surgically prepared as described for experiment 1 except that the radiotelemetered thermistor was not implanted. The head was fixed in a stereotactic frame. A midline scalp incision was made. A left-sided burr hole (2 mm diameter) was drilled and the dura opened. All drilling was performed under an operating microscope using a high-speed drill. The site was irrigated with normal saline to avoid thermal trauma. A glass microelectrode (tip diameter, \( \approx 5 \mu m \); intraparenchymal shaft diameter, \( \approx 20 \mu m \)) filled with 4 M NaCl and containing an Ag/AgCl wire was stereotactically positioned into the CA1 sector of the hippocampus (3.9 mm posterior and 2 mm left lateral of the bregma and 1.6 mm ventral to the cortical surface). The reference electrode was a Ag/AgCl disc (type E5SH; Grass Instruments, Quincy, MA) applied with electrode cream (EC2; Grass Instruments) to shaved skin on the animal’s neck. The DC potential between electrodes was recorded using a H105 high-impedance input probe attached to a 7P122 Low Level DC amplifier (Grass Instruments). Cortical EEG activity was recorded as described in experiment 1. A 22-gauge needle thermistor (model 524; YSI Co., Yellow Springs, OH) was placed percutaneously adjacent to the skull beneath the temporalis muscle, and pericranial temperature was servoregulated (model 73ATA indicating controller; YSI Co.) at \( 37.5 \pm 0.1^\circ C \) by surface heating or cooling.

After the anesthetic protocol described in experiment 1 for isoflurane-anesthetized rats, animals were subjected to either incomplete or near-complete forebrain ischemia (n = 4 per group). The EEG activity in the cortex and hippocampus was monitored for attenuation–isoelectricity. The hippocampal DC potential was monitored and the magnitude of shift (mV) was recorded. At the conclusion of the study, correct placement of the electrode in the hippocampus was confirmed histologically.

**Results**

**Experiment 1**

*Post hoc* analysis of EEG recordings caused the deletion of 4 of 15 fentanyl–nitrous oxide, 2 of 15 isoflurane, and 4 of 15 ketamine-treated rats in the respective incomplete ischemia groups because EEG isoelectricity was present. For near-complete ischemia, 4 of 15 fentanyl–nitrous oxide, 8 of 16 isoflurane, and 4 of 14 ketamine-treated rats were deleted from further analysis because of persistent EEG activity during the ischemic insult.

Tables 1 and 2 show physiologic values for the remaining animals in experiment 1. For incomplete ischemia, preischemic glucose was greater in the isoflurane group (\( P = 0.03 \)). Sixty minutes after ischemia, MAP was less in the isoflurane group (\( P = 0.002 \)). For near-complete ischemia, MAP at 10 min before ischemia (\( P = 0.03 \)) and 60 min after ischemia (\( P = 0.005 \)) was less in the isoflurane group. Brain temperature was controlled as intended. At all times, in either protocol, with
ANESTHETICS AND GLOBAL ISCHEMIA

Table 2. Physiologic Values for Near-Complete Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl/N₂O</th>
<th>Isoflurane</th>
<th>Ketamine</th>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td>10</td>
<td>8</td>
<td>10</td>
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<tr>
<td><strong>Preischemia</strong></td>
<td></td>
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<tr>
<td>Weight (g)</td>
<td>300 ± 23</td>
<td>286 ± 27</td>
<td>295 ± 15</td>
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<tr>
<td>Day 7 Weight (g)</td>
<td>260 ± 54</td>
<td>304 ± 37</td>
<td>308 ± 21</td>
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<tr>
<td><strong>10 min preischemia</strong></td>
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</tr>
<tr>
<td>MAP (mmHg)</td>
<td>123 ± 25</td>
<td>97 ± 11</td>
<td>127 ± 30</td>
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<tr>
<td>pH₅</td>
<td>7.38 ± 0.03</td>
<td>7.35 ± 0.03</td>
<td>7.38 ± 0.03</td>
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<tr>
<td>Pco₂ (mmHg)</td>
<td>40 ± 2</td>
<td>39 ± 1</td>
<td>39 ± 2</td>
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<tr>
<td>PaO₂ (mmHg)</td>
<td>124 ± 13</td>
<td>134 ± 19</td>
<td>130 ± 18</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>138 ± 33</td>
<td>149 ± 24</td>
<td>133 ± 29</td>
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<tr>
<td>Hematocrit (%)</td>
<td>45 ± 4</td>
<td>44 ± 2</td>
<td>45 ± 3</td>
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<tr>
<td><strong>10 min posts ischemia</strong></td>
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<tr>
<td>MAP (mmHg)</td>
<td>116 ± 31</td>
<td>125 ± 15</td>
<td>134 ± 21</td>
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<td>pH₅</td>
<td>7.26 ± 0.07</td>
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<tr>
<td>Pco₂ (mmHg)</td>
<td>38 ± 4</td>
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<td>PaO₂ (mmHg)</td>
<td>133 ± 25</td>
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<td>128 ± 15</td>
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<td><strong>60 min posts ischemia</strong></td>
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<td>MAP (mmHg)</td>
<td>103 ± 24</td>
<td>69 ± 17</td>
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<tr>
<td>pH₅</td>
<td>7.34 ± 0.06</td>
<td>7.37 ± 0.04</td>
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<tr>
<td>Pco₂ (mmHg)</td>
<td>40 ± 5</td>
<td>40 ± 4</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>113 ± 15</td>
<td>110 ± 15</td>
<td>114 ± 13</td>
</tr>
</tbody>
</table>

Values are mean ± SD. 
* Significant difference among groups (P < 0.05).

Among all animals, motor scores and hippocampal CA1 damage were inversely correlated (Kendall's τ = -0.27; P = 0.0025). Motor scores and CDI in the neocortex were inversely correlated (Kendall's τ = -0.35; P = 0.0007).

**Experiment 2**

Table 3 shows the results of the electrophysiologic study. All rats in the near-complete ischemia group exhibited isoelectric EEG and DC depolarization. Results

each of the three anesthetic regimens, the cortical temperature among the animals never varied significantly, at 37.5 ± 0.1 or 37.4 ± 0.1°C throughout, except for one episode of 37.2 ± 0.3°C 10 min after the onset of ischemia in the fentanyl–nitrous oxide group during near-complete ischemia. Figure 1 shows hippocampal injury. We did not detect a difference in the percentage of dead CA1 neurons (mean ± SD) among anesthetic agents during incomplete ischemia (fentanyl, 38 ± 20%; isoflurane, 31 ± 10%; ketamine, 40 ± 19%; P = 0.38). During near-complete ischemia, a difference existed among anesthetic agents (fentanyl, 88 ± 9%; isoflurane, 37 ± 20%; ketamine, 70 ± 28%; P = 0.00008). By post hoc analysis, isoflurane was protective compared with fentanyl (P = 0.000067) and ketamine (P = 0.0061). We did not detect a significant difference between fentanyl and ketamine (P = 0.143). For the cortex (fig. 2), we did not detect a difference in the crude damage index (median ± quartile deviation) among anesthetic agents during incomplete ischemia (fentanyl, 1 ± 0; isoflurane, 1 ± 0; and ketamine, 1 ± 0; P = 0.34). During near-complete ischemia, differences in total motor score existed among anesthetic agents (fentanyl, 2 ± 0; isoflurane, 1 ± 0; and ketamine, 2 ± 0; P = 0.02). The motor scores are depicted graphically in figure 3.

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A) Incomplete Ischemia

Crude Damage Index

Fentanyl/\textsubscript{N}_2\textsubscript{O}  Isoflurane  Ketamine

B) Near-complete Ischemia

Crude Damage Index

Fentanyl/\textsubscript{N}_2\textsubscript{O}  Isoflurane  Ketamine

Fig. 2. The severity of histologic damage in the neocortex after either incomplete (A) or near-complete (B) forebrain ischemia. Horizontal bars depict median values. Isoflurane caused reduced damage after near-complete ischemia when compared with either ketamine or fentanyl–nitrous oxide ($P = 0.02$). There was no effect for anesthetic on histologic outcome from incomplete ischemia. 0 = no observed histologic change; 1 = 1–5% neurons damaged; 2 = 6–50% neurons damaged; 3 = 51–100% of neurons damaged; and 4 = infarction.

from the incomplete ischemia study varied. Rat 2 (table 3) would have been deleted from the incomplete ischemia histology-neurology study had it presented with the cortical EEG isoelectricity, as indicated.

Discussion

This investigation shows that outcome from global cerebral ischemia, sufficiently severe to cause EEG isoelectricity, can be influenced by the anesthetic agent chosen. In contrast, during less severe ischemia, allowing persistent EEG activity, the anesthetic agents examined resulted in no outcome differences. These findings are inconsistent with our \textit{a priori} expectations that differential anesthetic effects on outcome would be observed only under conditions in which EEG activity persisted during ischemia. Accordingly, both the mod-

A) Incomplete Ischemia

Total Motor Score

Fentanyl/\textsubscript{N}_2\textsubscript{O}  Isoflurane  Ketamine

B) Near-complete Ischemia

Total Motor Score

Fentanyl/\textsubscript{N}_2\textsubscript{O}  Isoflurane  Ketamine

Fig. 3. Total motor score as determined 5 days after either incomplete (A) or near-complete (B) forebrain ischemia as a function of anesthetic agent. Each open circle depicts that value for a single rat. Horizontal bars denote the median value for each group. 9 = no deficits.
els that we used in the current study and the theoretical basis for our expectations must be considered.

The current study was performed in the context of rigidly controlled brain temperature known to be a critical determinant of ischemic outcome. Mean arterial pressure in the isoflurane group was less than that of the other groups at several stages of the experimental protocol. This is unlikely to be important. It is difficult to speculate how modest preischemic differences in MAP would alter outcome when the intraischemic MAP was held identical among groups. Furthermore, during early reperfusion, it has been shown that substantially greater reductions in MAP (i.e., sustained decreases to 25 mmHg) are required to alter CA1 damage. No differences among groups for plasma glucose were observed in the near-complete ischemia study, and differences in the incomplete ischemia study were modest and substantially lower than differences required to result in measurable differences in ischemic outcome, as previously documented in this rodent model. The recovery interval of 5 days was sufficient to ensure that peak delayed neuronal necrosis had occurred. Furthermore, only those animals that strictly met criteria for either cortical EEG isoelectricity (near-complete ischemia) or attenuated cortical EEG activity (incomplete ischemia) were considered in the respective analyses. Accordingly, it is difficult to ascribe the findings of this study to any technical errors that are known to influence ischemic outcome.

The findings of this study are both supported and contradicted by other reports in the literature. In 1973, Michenfelder and Theye subject fasted dogs breath-
Two methodologic features readily distinguish the current experiment from our earlier work. First, the importance of rigid control of either pericranial or brain temperature was not recognized in 1986. Instead, rectal temperature was monitored and controlled only during the 10 min of global ischemia. Subsequent studies proved that control of rectal temperature provides little assurance that brain temperature is controlled and that only small differences in brain temperature are critical in defining global ischemic outcome. In contrast, the current experiment provided 22 h of brain temperature control. The question of a potential confounding effect of unregulated brain temperature may itself be sufficient to allow the early work by Warner et al. to be disregarded. Second, the current study used no sympathetic ganglionic blocking agent. In contrast, Warner et al. used trimethaphan to facilitate a rapid onset of systemic hypotension at the onset of ischemia. Use of ganglionic blocking agents has been questioned with respect to presenting potential confounding effects in models of global cerebral ischemia and warrants careful consideration.

It can be theorized that differences in depth and character of anesthesia between the isoflurane and nitrous oxide-fentanyl-treated rats in the current experiment can account for the marked differences in outcome from near-complete ischemia. If true, we must ask how differences in depth of anesthesia could be important. One hypothesis is that the two anesthetic states resulted in different adrenergic responses to the hemorrhagic hypotension used to create the ischemic insult. This is supported by the relatively lower MAP observed in the isoflurane-treated animals at different peri-ischemic observation intervals. If adrenergic events during ischemia–reperfusion are important and modulated by anesthetic state, then use of trimethaphan could mask the effect of anesthesia on ischemic outcome. Because rats were not given trimethaphan in the current study, anesthetic effects may then have become manifest as differences in histologic–behavioral outcome.

The effect of adrenergic events on outcome from forebrain ischemia is controversial. Koide et al. reported that use of trimethaphan in this model increased histologic damage. Co-administration of exogenous catecholamines reversed the effect of trimethaphan and improved outcome. Further work supported the finding that increased catecholamine concentrations are neuroprotective. In contrast, Werner et al. found that sympathetic ganglionic blockade, achieved with hexamethionium, resulted in reduced ischemic injury. Co-administration of exogenous catecholamines reversed the beneficial effect of ganglionic blockade and worsened histologic outcome. Further work supported the conclusion that a reduction of catecholamines is neuroprotective. Neither of these apparently contradictory sets of observations were obtained under conditions of brain temperature control. To date, no effort has been made to resolve the controversy surrounding the effect of circulating catecholamines on ischemic outcome.

We can only speculate that adrenergic influences of different anesthetic states are important in explaining the differential effects of anesthetic agents observed in the current study. Neither catecholamine concentrations nor postischemic glucose concentrations were measured, simply because we did not anticipate an effect of anesthetic agent on near-complete ischemia. We believe, however, that the results of the current experiments dictate a close examination of the role of catecholamines on ischemic outcome and of how the choice of anesthetic agent might affect that interaction.

We were also surprised to find no effect of anesthetic agent on outcome from the less severe incomplete ischemia. Several factors may account for this, although we speculate that a primary consideration is the variability of ischemic insult. Cerebral blood flow studies of the near-complete insult causing EEG isoelectricity have consistently found flow to be severely reduced (i.e., to a level <5% of control). In contrast, examination of cerebral blood flow during less severe forebrain ischemia (i.e., where greater variability of EEG activity was allowed) found substantial scatter in the magnitude of cerebral blood flow reduction. It is clear from our current observations made in experiment 2 that the near-complete ischemia insult was uniformly severe across all animals. Cortical and hippocampal EEG recordings were homogeneous with respect to the presence or absence of isoelectricity, and all animals underwent depolarization. In contrast, findings in the incomplete model varied with respect to concordance between cortical and hippocampal EEG as well as between the presence or absence of EEG activity and the presence or absence of DC depolarization. These differences presumably were present in animals subjected to incomplete ischemia in experiment 1 and may have introduced sufficient variability in insult severity to obscure any potential relative protective effect of the various anesthetic regimens. We speculate that the incomplete ischemia model may be improved by using a targeted change in processed EEG parameters rather than MAP as a physiologic end point.
Extrapolation of our current work to clinical practice is limited both because of the species examined and because our findings should be confirmed in other global ischemia models. However, other recent work using temperature-controlled focal ischemia models has provided rather surprising results with respect to the protective effects of volatile anesthetic agents. Both halothane and isoflurane have been shown to cause marked reduction in infarct size in rats subjected to transient focal cerebral ischemia. This has been confirmed and extended in a cat model of permanent middle cerebral artery occlusion. Minimally, this accumulating body of evidence indicates a careful reevaluation of the effects of volatile anesthetic agents on ischemic brain damage.

In conclusion, rats were subjected to an ischemic insult designed to cause either attenuation of EEG activity or isoelectricity. Three different anesthetic states were examined with respect to the relative effects on outcome as determined by both histologic and behavioral analyses. Relevant physiologic values were adequately controlled. In rats allowed to maintain persistent EEG activity during ischemia, a significant differential effect of anesthetic agents was absent. In contrast, in animals sustaining an insult severe enough to cause EEG isoelectricity, those that were anesthetized with isoflurane had less damage than did rats given either ketamine or fentanyl-nitrous oxide. It was suggested that differences in the depth and character of anesthesia and associated influences on catecholamine concentrations might account for the observed effects of choice of anesthetic in this global ischemia model.

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References

2. Michenfelder JD, Theye RA: Cerebral protection by thiopental during hypoxia. ANESTHESIOLOGY 1973; 39:510-7
10. Michenfelder JD: The interdependency of cerebral function and metabolic effects following massive doses of thiopental in the dog. ANESTHESIOLOGY 1974; 41:251-6
23. Gionet TX, Warner DS, Verhaegen M, Thomas JD, Todd MM:


32. Baughman VL, Hoffman WE. Neurologic outcome in rats following incomplete cerebral ischemia during halothane, isoflurane, or N2O. Anesthesiology 1988; 69:192-8


40. Schultz JIA, Hoffman WE, Albrecht RF. Sympathetic stimulation with phystostigmine worsens outcome from incomplete brain ischemia in rats. Anesthesiology 1993; 78:114-21


42. Warner DS, Ludwig PS, Pearlestein R, Brinkhaus AD. Halothane reduces focal ischemic injury in the rat when brain temperature is controlled. Anesthesiology 1995; 82:1237-45


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