

Anesthetic Effects on Cerebral Metabolic Rate Predict Histologic Outcome from Near-complete Forebrain Ischemia in the Rat

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Background: Although reduction of cerebral metabolic rate is thought to contribute to anesthetic neuroprotection, histologic evidence to support this concept has not been provided. In this study, histologic outcome was evaluated in rats subjected to different durations of severe forebrain ischemia while anesthetized with volatile anesthetics that have substantially different effects on cerebral metabolic rate.

Methods: Normothermic rats that underwent fasting were anesthetized with 0.75 minimum alveolar concentration (MAC) isoflurane–60% nitrous oxide (N₂O) or 0.75 MAC halothane–60% N₂O. Ischemia was induced with use of a combination of bilateral carotid occlusion and controlled hypotension. Rats in the isoflurane group were subjected to 6.5 min or 8.0 min

ischemia, whereas the halothane group received 6.5 min ischemia. Histologic damage was assessed 4 days later.

Results: With 6.5 min ischemia, mean \pm SD, hippocampal CA1 percent of dead (% dead) neurons was reduced with isoflurane–N₂O (45 \pm 18) versus halothane–N₂O (60 \pm 23, $P = 0.023$). Eight minutes of ischemia increased % dead neurons in the isoflurane–N₂O group (60 \pm 17, $P = 0.017$). There was no difference between the isoflurane 8.0-min and halothane 6.5-min groups ($P = 0.935$). A similar pattern was observed in hippocampal CA4 and the neocortex. Striatal damage was not affected by anesthetic or ischemic duration.

Conclusions: At 6.5 min ischemia, isoflurane provided improved outcome *versus* halothane. Previous research has shown that 0.75 MAC isoflurane–N₂O increases the time to onset of ischemic depolarization by 1.5 min and reduces cerebral metabolic rate by 42% *versus* 0.75 MAC halothane–N₂O. In the current study, when the duration of ischemia was increased by 1.5 min in the isoflurane–N₂O group, histologic outcome became similar to that in halothane–N₂O-anesthetized rats. These results provide evidence that cerebral metabolic rate reduction has an advantageous effect on outcome from severe brain ischemia, but also suggest that such benefit is likely to be small. (Key words: Depolarization; hippocampus; neocortex.)

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ISOFLURANE is known to produce major reduction of cerebral metabolic rate (CMR).¹ This property has been proposed to provide protection against ischemic brain injury.² Early biochemical evidence suggested that isoflurane is neuroprotective.³ However, subsequent laboratory studies compared histologic–neurologic outcome from ischemic injury in animals anesthetized with isoflurane *versus* other anesthetic agents which provide lesser degrees of CMR reduction and failed to provide evidence for superiority of isoflurane.^{4–6} This cumulative outcome evidence was considered to be sufficient to conclude that anesthetic effects on CMR cannot predict effectiveness of anesthetics as neuroprotective agents.⁷

However, some information remained unaccounted for. First was the simple logic that when brain adenosine triphosphate is depleted, dissipation of ionic gradients occurs, followed by massive influx of calcium into the neuron.^{8,9} The time to onset of this event, defined on the

basis of a shift in brain parenchymal direct current (DC) potential, is known to be related to preischemic CMR.^{10,11} This evidence allowed prediction that anesthetics with different potency in reducing CMR could be associated with different times to loss of direct current potential (*i.e.*, time to depolarization). Indeed, Verhaegen *et al.*¹² observed a 1.5 min delay in time to depolarization in rats subjected to near-complete forebrain ischemia injury while anesthetized with 0.75 minimum alveolar concentration (MAC) isoflurane-60% nitrous oxide (N₂O) *versus* 0.75 MAC halothane-60% N₂O. At these doses, cortical CMR of glucose (CMR_{glucose}) was 42% less in the isoflurane-anesthetized rats.

The significance of anesthetic-mediated CMR reduction and its associated effect on time to ischemic depolarization has not been evaluated in an outcome model. We designed an experiment that precisely replicated the experimental conditions used by Verhaegen *et al.*¹² Rats anesthetized with equi-MAC doses (*i.e.*, 0.75 MAC) of either halothane or isoflurane in 60% N₂O-balance oxygen were subjected to forebrain ischemia. Ischemia duration was varied to allow the duration of postdepolarization ischemia to be equivalent for the two anesthetics, thereby segregating time to depolarization (and therefore CMR) as an outcome variable. Histologic damage was evaluated 4 days later.

Materials and Methods

This study was approved by the Duke University Animal Care and Use Committee. Male Sprague-Dawley rats (8-10 weeks, Harlan Sprague-Dawley, Indianapolis, IN) underwent fasting for 12-16 h, but were allowed free access to water. The animals were then anesthetized with 3-5% halothane or isoflurane in oxygen. After orotracheal intubation, the lungs were mechanically ventilated (30-40% O₂-balance nitrogen [N₂]). The inspired concentration of halothane or isoflurane (1 to 2%) was adjusted to maintain mean arterial pressure (MAP) within 80-120 mmHg. Surgery was performed using an aseptic technique, and all wounds were infiltrated with 1% lidocaine. The tail artery was cannulated. *Via* a neck incision, the right jugular vein was cannulated. The common carotid arteries were exposed and encircled with sutures, leaving the vagus nerves and cervical sympathetic plexus intact.

Electroencephalography (EEG) was monitored with use of active subdermal electrodes positioned over the

parietal cortex bilaterally. A 22-gauge needle thermistor was placed adjacent to the skull beneath the temporalis muscle, and pericranial temperature was servoregulated at 37.0 ± 0.1°C with surface heating (heat lamp) or cooling during ischemia and for 2 h after recirculation. Heparin (50 IU) was given intravenously.

The animals were randomly assigned to one of three groups (n = 20 per group):

Halothane 6.5 min: Halothane anesthesia was continued

The inspired dose was reduced to 0.8% in 60% N₂O-balance oxygen. The duration of forebrain ischemia was 6.5 min;

Isoflurane 6.5 min: Isoflurane anesthesia was continued

Isoflurane 1.1% was inhaled in 60% N₂O-balance oxygen. The duration of forebrain ischemia was 6.5 min;

Isoflurane 8.0 min: Isoflurane anesthesia was continued

Isoflurane 1.1% was inhaled in 60% N₂O-balance oxygen. The duration of forebrain ischemia was 8.0 min.

A 30-min interval of physiologic stabilization was provided in all animals to allow anesthetic stabilization and to adjust minute ventilation to produce normocapnia. Muscle paralysis was provided by intravenous rocuronium, 3.5 mg/kg, repeated when necessary.

Near-complete forebrain ischemia was induced by withdrawal of blood from the jugular venous catheter to maintain mean arterial pressure at 30 ± 5 mmHg, concomitant with bilateral carotid artery occlusion using aneurysm clips.^{13,14} *Ischemia* was defined as the time between clip placement and removal. To discontinue ischemia, the shed blood was reinfused and the carotid clips were removed. Intravenous NaHCO₃ (0.3 mEq) was administered to minimize systemic acidosis.

The anesthetic states and pericranial temperature regulation were continued for an additional 2 h. Anesthetics were then discontinued. At recovery of the righting reflex, animals were extubated and placed in an oxygen-enriched environment (approximately 50% O₂-50% N₂) for 1 to 2 h. Animals were then returned to their home cages.

On the fourth postischemic day, with the observer blind to group assignment, motor function tests were performed according to an established protocol, including assays of prehensile traction and balance beam performance.^{15,16} The motor score was graded on a scale of 0-9 (best score = 9).

The rats were then anesthetized with halothane in oxygen-nitrogen and perfused *in situ* with buffered 10%

formalin. Paraffin-embedded brain sections were serially cut (5- μ m thick) and stained with acid fuchsin-celestine blue. With the investigator blind to group assignment, all viable and necrotic neurons in the CA1 sector of hippocampus (bregma -4.0) were counted and the % dead neurons was calculated. Hippocampal CA4 damage was evaluated at the same coronal level and graded on a scale of 1-4, (1 = 0-25% of neurons damaged; 2 = 25-50% of neurons damaged; 3 = 50-75% of neurons damaged and 4 = 75-100% of neurons damaged). Damage in the striatum was evaluated in a lateral circular area of 400 μ m in diameter, at a level 0.3 mm caudal to bregma. Viable and necrotic neurons were counted and damage is presented as % dead neurons. Neocortical damage was also evaluated in a circular area with a 400- μ m diameter dorsal to the entorhinal fissure 3.8-4.0 mm caudal to bregma. The total of necrotic neurons was counted and is presented as the number of necrotic neurons/high-power field.

Statistical Analysis

Parametric values, including histologic injury in the hippocampal CA1, neocortex, and striatum, were subjected to analysis of variance. When indicated by a significant F ratio, between-group differences were defined using *post hoc* protected least-squares difference analysis. Values are reported as mean \pm SD. Nonparametric values (*e.g.*, motor function and hippocampal CA4 damage scores) were compared with use of the Kruskal-

Wallis H statistic and are reported as median \pm interquartile deviation. Significance was assumed when $P < 0.05$.

Results

One rat died in the halothane 6.5-min group from undefined causes. Physiologic values for survivors are reported in table 1. Values were similar among experimental groups. Motor function scores were also similar among groups (halothane 6.5 min = 7 ± 4 ; isoflurane 6.5 min = 7 ± 2 ; isoflurane 8.0 min = 7 ± 2 , $P = 0.24$).

Histologic injury is presented in figure 1. Hippocampal CA1 damage was different among groups ($P = 0.03$). With 6.5 min ischemia, damage was reduced in isoflurane- (45 \pm 18 % dead neurons) *versus* halothane-anesthetized rats (60 \pm 23 % dead neurons, $P = 0.02$). Extension of the duration of ischemia to 8.0 min increased CA1 damage in isoflurane-anesthetized rats (60 \pm 17 % dead neurons, $P = 0.02$). There was no difference between the isoflurane 8.0-min and halothane 6.5-min groups ($P = 0.94$). A similar pattern was observed in CA4.

Damage in the neocortex was also different among groups ($P = 0.03$). The number of necrotic neurons/high-power field was less in the isoflurane 6.5-min group (27 \pm 12 necrotic neurons/high-power field) compared with the halothane 6.5-min group (37 \pm 12, $P = 0.02$). Extension of ischemia duration to 8.0 min increased the number of neocortical necrotic neurons in isoflurane-

Table 1. Physiologic Values in Rats Subjected to Forebrain Ischemia

	Halothane, 6.5 min	Isoflurane, 6.5 min	Isoflurane, 8.0 min
Preischemia body weight (g)	295 \pm 13	292 \pm 12	292 \pm 216
Day 4 body weight (g)	269 \pm 27	300 \pm 19	285 \pm 29
10 min preischemia			
MAP (mmHg)	90 \pm 7	99 \pm 13	106 \pm 12
Arterial pH	7.36 \pm 0.04	7.37 \pm 0.03	7.35 \pm 0.03
Pa _{CO₂} (mmHg)	38 \pm 3	36 \pm 3	39 \pm 3
Pa _{O₂} (mmHg)	124 \pm 18	148 \pm 23	145 \pm 22
Glucose (mg/dl)	105 \pm 8	116 \pm 16	112 \pm 16
Hematocrit concentration (%)	40 \pm 2	39 \pm 1	39 \pm 1
Pericranial temperature (°C)	37.0 \pm 0.1	37.0 \pm 0.1	37.0 \pm 0.1
10 min postischemia			
MAP (mmHg)	104 \pm 9	104 \pm 11	104 \pm 13
Arterial pH	7.40 \pm 0.03	7.39 \pm 0.03	7.37 \pm 0.03
Pa _{CO₂} (mmHg)	37 \pm 2	39 \pm 2	40 \pm 2
Pa _{O₂} (mmHg)	128 \pm 19	153 \pm 44	139 \pm 22
Glucose (mg/dl)	108 \pm 10	125 \pm 11	117 \pm 15
Hematocrit concentration (%)	39 \pm 1	39 \pm 1	39 \pm 1
Pericranial temperature (°C)	37.0 \pm 0.1	37.0 \pm 0.1	37.0 \pm 0.1

Mean \pm SD. MAP = mean arterial pressure; Pa_{CO₂} = arterial blood carbon dioxide partial pressure; Pa_{O₂} = arterial blood oxygen partial pressure; N = 19-20 per group.

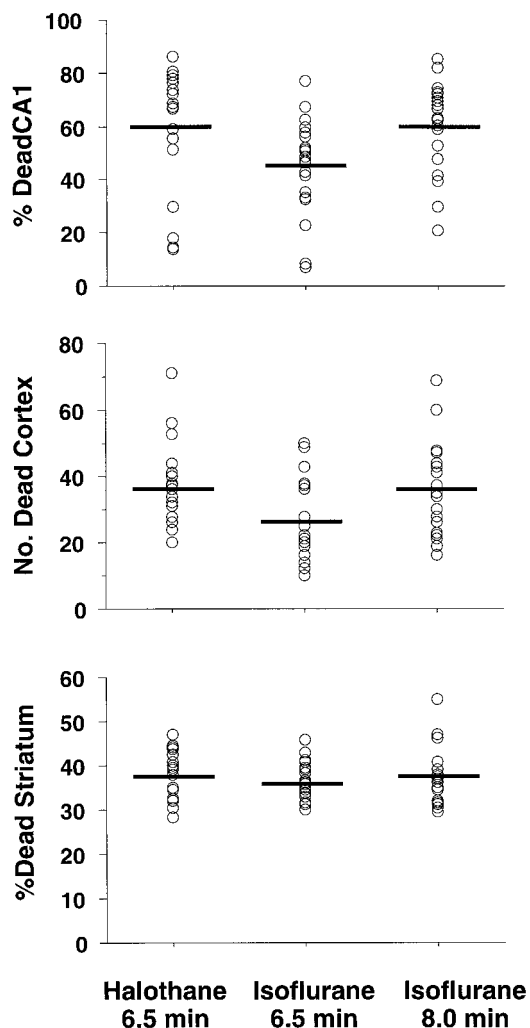


Fig. 1. Histologic damage resulting from near-complete forebrain ischemia in rats anesthetized with 1.1% (0.75 MAC) isoflurane–60% nitrous oxide (N₂O) versus 0.8% (0.75 MAC) halothane–60% N₂O. After 6.5 min ischemia, isoflurane-anesthetized rats had less damage in the hippocampal CA1 ($P = 0.02$) and neocortex ($P = 0.02$) than did halothane-anesthetized rats subjected to 6.5 min ischemia. Extension of the ischemic interval to 8.0 min in isoflurane-anesthetized rats resulted in increased CA1 ($P = 0.02$) and neocortical ($P = 0.03$) damage relative to the isoflurane 6.5-min group, but damage was equivalent to that observed in the halothane 6.5-min ischemia group. There was no difference in the striatum between groups. Open circles represent values for individual animals. Horizontal bars depict group mean. $n = 19$ or 20 animals/group.

anesthetized rats (36 ± 14 , $P = 0.03$). There was no difference between the isoflurane 8.0-min and halothane 6.5-min groups ($P = 0.83$).

There was no difference among groups for % dead neurons in the striatum (halothane 6.5 min = 39 ± 5 ;

isoflurane 6.5 min = 37 ± 4 ; and isoflurane 8.0 min = 39 ± 8 ; $P = 0.54$).

Discussion

In three of four structures evaluated, extension of ischemia from 6.5 to 8.0 min increased histologic damage in isoflurane-anesthetized rats. Although rats anesthetized with isoflurane incurred less damage than did those anesthetized with halothane during 6.5 min ischemia, extension of the ischemic interval to 8.0 min in the isoflurane group caused damage to be equivalent to the halothane 6.5-min group.

The 1.5-min difference in duration of ischemia was selected to capture the difference in time to depolarization measured for the two anesthetics by Verhaegen *et al.*,¹² who studied an identical ischemic injury. In that study, a 1.5-min greater delay in time to depolarization was associated with a 42% lower $CMR_{glucose}$ in the isoflurane–N₂O versus halothane–N₂O groups. When that study was performed, it was thought that such a small delay in onset of ischemic depolarization could not be detected as a difference in histologic outcome because of limitations in model sensitivity. Accordingly, a test of the relevance of anesthetic effects on CMR and time to depolarization was not pursued.

Recently, we evaluated rats subjected to either 7 or 8 min of a similar forebrain ischemic injury while attempting to define the impact of preischemic brain norepinephrine depletion on ischemic outcome.¹⁷ Control groups, having normal brain norepinephrine concentrations, were found to have a statistically worsened CA1 damage, resulting from the 8- versus 7-min injury (7 min = 57 ± 34 % dead neurons; 8 min = 78 ± 2 % dead neurons, $P = 0.041$). That information, along with recent recurrent evidence that isoflurane reduces focal and global ischemic brain injury when compared to other anesthetics,^{18–20} gave reason to revisit the question of the influence of CMR reduction on ischemic outcome.

The results of the current experiment can be held in contrast to another study that used a similar experimental design. Hypothermia is also known to reduce CMR and the time to depolarization in ischemic brain.²¹ It can be postulated that one mechanism by which hypothermia reduces ischemic brain damage is by decreasing the duration of depolarization (and therefore the duration of calcium influx) during the ischemic injury. To test whether this delay in onset of depolarization has an influence on outcome, rats were subjected to ischemia

of varied durations to make the duration of normothermic intranschemic depolarization equivalent to the duration of hypothermic intranschemic depolarization.²² Although the total duration of ischemia was 4 min longer in the hypothermic rats (*i.e.*, 14 *versus* 10 min), histologic damage was still markedly less severe (*i.e.*, near zero) than that observed in normothermic rats. In contrast to the case for isoflurane, these results indicate that hypothermic protection relies on far more important neuroprotective mechanisms than simple reduction of CMR.

Because of recent evidence that isoflurane and other volatile anesthetics are neuroprotective,^{18,20,23} there is renewed interest in defining the mechanisms by which such protection occurs. Volatile anesthetic agents have been identified as effective *N*-methyl-D-aspartate (NMDA) antagonists.²⁴⁻²⁷ Activation of this receptor allows calcium entry. It remains plausible that a principle cause of ischemic injury is overexcitation of this receptor by a massive increase in extracellular glutamate concentration.²⁸ This increase has been shown to occur at blood flow thresholds less than those necessary for onset of ischemic depolarization.²⁹ We speculate that isoflurane (and potentially other volatile anesthetics) serves a dual action by delaying depolarization-mediated increases in glutamate concentration and then by inhibiting the NMDA receptor when glutamate release eventually occurs. This speculation would also predict that halothane and isoflurane would be equally effective in inhibiting NMDA neurotoxicity *in vitro*, a test that to date has not been performed. Of course, when considering mechanistic neuroprotective actions, potential interactions with γ -aminobutyric acid-mediated (GABAergic) properties of volatile anesthetic agents cannot be overlooked.^{30,31}

There have been no appropriately designed human trials that allow direct comparison of the relative neuroprotective effects of volatile anesthetics. However, two human studies have evaluated the relative electrophysiologic effects of approximately 0.75 MAC isoflurane *versus* halothane in patients also receiving N₂O while undergoing carotid endarterectomy.^{32,33} That work showed a differential cerebral blood flow reduction necessary for ischemic changes to become evident on the electroencephalogram. Flow thresholds for EEG changes were lower with isoflurane. In one of those studies, neurologic outcome was reported and there was no effect of anesthetic agent.³² Although methodologic factors limit interpretation (*i.e.*, retrospective design and non-current study of the different anesthetics), absence

of a differential outcome does not rule out a potentially greater protective effect of isoflurane. Flow thresholds necessary for EEG changes are substantially greater than those necessary for release of neurotransmitters or neuronal depolarization.³⁴⁻³⁶ The results of our study suggest that differential effects of halothane and isoflurane on outcome might only be found when the lower flow thresholds are reached and would only be relevant to situations in which the duration of ischemia is brief enough to allow the respective anesthetic effects on time to depolarization to become important.

In conclusion, we replicated conditions of a previous study in the rat in which 0.75 MAC isoflurane-60% N₂O was found to cause an 1.5-min delay in time to onset of ischemic depolarization, compared with 0.75 MAC halothane-60% N₂O. When both anesthetic groups underwent 6.5 min of severe forebrain ischemia, better histologic outcome was observed in the isoflurane group. When the duration of ischemia was extended in the isoflurane group, so that the duration of ischemic depolarization was nearly identical to that of the halothane group, the advantage of isoflurane was lost. These results provide evidence that CMR reduction has an advantageous effect on outcome from severe brain ischemia, but also suggest that such benefit is likely to be small.

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