Minimum Alveolar Concentration for Halothane in the Rat Is Resistant to Effects of Forebrain Ischemia and Reperfusion
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Background: Because glutamate antagonists can substantially alter the minimum alveolar concentration (MAC) of volatile anesthetics, glutamate has been implicated as an important neurotransmitter in processes contributing to the anesthetic state. Cerebral ischemia profoundly alters glutamatergic neurotransmission and thus may also alter halothane MAC.

Methods: Fasted rats were surgically prepared for forebrain ischemia. One half of the animals served as operative shams (n = 24). Remaining rats underwent 10 min of bilateral carotid occlusion combined with systemic hypotension (n = 24). In animals in both groups brain circulation was restored for 1 h, 24 h, or 7 days (n = 8). At each of these intervals, MAC for halothane was determined by the tail-clamp method. Histologic damage in the hippocampus, cortex, caudoputamen, and thalamus was measured in animals allowed to survive 7 days.

Results: Blood pressure, arterial blood gas tensions and pH, pericranial temperature, and plasma glucose values measured immediately before ischemia were similar among groups. Neither ischemia nor duration of reperfusion significantly altered halothane MAC as compared with operative shams (sham 1 h = 0.9 ± 0.1 vol%, 24 h = 1.0 ± 0.1 vol%, 7 days = 1.0 ± 0.2 vol%; ischemia 1 h = 0.9 ± 0.1 vol%, 24 h = 0.9 ± 0.1 vol%, 7 days = 1.0 ± 0.2 vol%). Histologic damage in the hippocampus and caudoputamen was severe in the ischemic group of animals. Mild injury was observed in the motor and cingulate cortex as well as the thalamus. There was no evidence of histologic injury in sham-operated animals.

Conclusions: The absence of effect of ischemic forebrain injury on halothane MAC is consistent with findings made in other models of supratentorial cerebral injury. These results support the contention that anatomic foci for motor responses elicited during MAC determination are localized at levels caudal to the forebrain. (Key words: Anesthetics, volatile; halothane; minimum alveolar concentration. Animals: rat. Brain: ischemia.)

Numerous investigators have demonstrated that selective pharmacologic antagonism of glutamatergic neurotransmission profoundly decreases the minimum alveolar concentration (MAC) for volatile anesthetic agents.1–4 These findings implicate glutamate as a principle neurotransmitter in neural networks underlying some behavioral responses to peripheral noxious stimuli.

Cerebral ischemia is known to grossly perturb glutamatergic neurotransmission. For example, extracellular concentrations of glutamate may increase four- to tenfold during a dense global insult.5–7 Although the concentration of extracellular glutamate is restored to normal soon after reperfusion of the brain, experimental evidence indicates that postsynaptic sensitivity to normal extracellular concentrations of glutamate transiently increases, perhaps because of DNA transcriptional events causing changes in glutamate receptor affinity or coupled ionotropic conductance.8 Such findings may explain the hyperexcitability and frequent seizures observed at approximately 24 h after the global insult.9 Days later, many neurons exposed to excitotoxic concentrations of glutamate necrose potentially reducing overall efficiency of glutamatergic neurotransmission.10,11 For these reasons, we hypothesized that rats undergoing a transient but severe forebrain ischemic insult would demonstrate a reperfusion interval-dependent change in the MAC for halothane.

Materials and Methods

This study was approved by the University of Iowa Animal Care and Use Committee. Forty-eight male
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Sprague-Dawley rats (body weight 300–325 g; Harlan, Indianapolis, IN) were fasted for 12–16 h before the experiment. All rats were anesthetized with 4% halothane in O₂. The tracheas were intubated and the lungs mechanically ventilated with a delivered mixture of 1.0–1.6% halothane in 30% O₂–balance N₂. The tail artery was cannulated and mean arterial pressure continuously recorded. Via a ventral neck incision, the common carotid arteries were isolated and encircled with suture. The right internal jugular vein was cannulated and heparin (35 IU) was given intravenously. Muscle paralysis was obtained from a 0.2 mg intravenous bolus of succinylcholine administered at completion of the surgical procedure and repeated immediately before the onset of ischemia. Bipolar electroencephalographic activity was monitored from active needle electrodes positioned in the temporalis bilaterally, a reference lead in the prefrontal region, and a ground lead in the tail. Finally, a 22-G needle thermistor (524, Yellow Springs Instruments, Yellow Springs, OH) was percutaneously placed adjacent to the skull and pericranial temperature was servoregulated at 37.0 ± 0.1°C by surface heating or cooling. The inspired halothane concentration was reduced to 0.5%, and 30 min was allowed for physiologic stabilization.

Rats were randomly assigned to undergo 10 min of forebrain ischemia or to serve as operative shams. The protocol for producing forebrain ischemia has been previously described. Hypotension (mean arterial pressure 30 ± 5 mmHg) was induced with trimethaphan (1.75 mg intravenously) and maintained by withdrawal and reinfusion of blood through the jugular catheter as necessary. Immediately after onset of hypotension, the carotid arteries were occluded bilaterally with temporary aneurysm clips. Ten minutes later, the vascular clamps were released and any shed blood was reinfluenced. NaHCO₃ (0.27 mEq) was given to minimize systemic acidosis. Sham-operated rats did not receive trimethaphan and did not undergo carotid occlusion or withdrawal of blood, but were otherwise treated identically to animals undergoing the ischemic insult.

After completion of the ischemia procedure or sham operation, rats were randomly assigned to one of three groups (n = 8). Group assignment was defined by the interval between reperfusion of the forebrain (or sham procedure) and determination of MAC for halothane (1 h, 24 h, or 7 days). Animals in the 1-h group remained anesthetized for the intervening 1 h with delivered halothane concentration adjusted to maintain mean arterial pressure at 90–120 mmHg (≈0.8 vol%). Mechanical ventilation and monitoring of arterial blood pressure continued. For rats in the 24-h and 7-day groups, the arterial and venous catheters were removed and incision sites were closed with suture. Approximately 20 min after the ischemic insult, halothane anesthesia was discontinued and the animals were awakened. On recovery of spontaneous ventilation, the tracheas were extubated. Rats were then placed in a chamber containing 50% O₂–50% N₂ for approximately 30 min and then were returned to their cages.

At respective postschematic (or sham-operation) intervals, rats in the 24-h and 7-day groups were reanesthetized with halothane and their tracheas were intubated. Mechanical ventilation of the lungs was performed with an end-tidal halothane concentration of 1.3% in 30% O₂–balance N₂. In the 1-h groups, the halothane concentration was also adjusted to 1.3%. The MAC for halothane was then determined using an established protocol. The painful stimulus was a rubber-shod 25-cm hemostat clamped to the proximal 2 cm of the tail. The clamp was applied for 60 s, during which time the hemostat was continually rotated across its long axis to simulate a wagging motion of the tail. A sustained movement of any of the four extremities during application of the hemostat was considered a positive response.

In the absence of movement, the inspired halothane concentration was reduced by approximately 20% (e.g., to 1.1%). End-tidal halothane concentration was recorded 20 min after ventilation at the new inspired halothane concentration. The stimulus was then repeated. If the animal failed to respond, the inspired halothane concentration was decreased again by 20% and the sequence was repeated until purposeful movement was observed. The value of the lowest end-tidal concentration at which the rat did not move and that end-tidal value at which movement was observed were averaged and recorded. Because recovery intervals were different among groups, the degree of wound healing prohibited blinding the observer to that aspect of the procedure. However, the observer (someone other than the experimenter inducing the ischemic insult) was blinded to whether the animal had undergone ischemia or only the sham operation.

End-tidal halothane concentrations were determined as follows. A polyethylene catheter was permanently positioned at the distal tip of the endotracheal tube. Small aliquots (≈1 ml for each ventilatory cycle) of expiratory gas were withdrawn into a glass syringe for

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Table 1. Physiologic Values for Rats Undergoing Halothane MAC Determination at Various Intervals after Forebrain Ischemia or Sham Operation

<table>
<thead>
<tr>
<th></th>
<th>1 Hour</th>
<th></th>
<th>24 Hours</th>
<th></th>
<th>7 Days</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sham (n = 8)</td>
<td>Ischemia (n = 8)</td>
<td>Sham (n = 8)</td>
<td>Ischemia (n = 8)</td>
<td>Sham (n = 8)</td>
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<tr>
<td>5 min preischemia</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>MAP (mmHg)</td>
<td>105 ± 11</td>
<td>108 ± 10</td>
<td>104 ± 17</td>
<td>110 ± 12</td>
<td>101 ± 15</td>
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<tr>
<td>pH₄</td>
<td>7.37 ± 0.04</td>
<td>7.36 ± 0.05</td>
<td>7.37 ± 0.04</td>
<td>7.33 ± 0.06</td>
<td>7.34 ± 0.07</td>
</tr>
<tr>
<td>P₈CO₂ (mmHg)</td>
<td>38 ± 4</td>
<td>36 ± 2</td>
<td>39 ± 3</td>
<td>38 ± 3</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>P₈O₂ (mmHg)</td>
<td>156 ± 21</td>
<td>153 ± 38</td>
<td>161 ± 2</td>
<td>176 ± 28</td>
<td>164 ± 30</td>
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<td>Glucose (mg/dl)</td>
<td>139 ± 29</td>
<td>124 ± 13</td>
<td>137 ± 13</td>
<td>131 ± 15</td>
<td>123 ± 21</td>
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<tr>
<td>Hematocrit (%)</td>
<td>43 ± 1</td>
<td>43 ± 3</td>
<td>44 ± 3</td>
<td>42 ± 3</td>
<td>43 ± 3</td>
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<td>10 min after reperfusion</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>MAP (mmHg)</td>
<td>95 ± 20</td>
<td>121 ± 14</td>
<td>92 ± 11</td>
<td>129 ± 10</td>
<td>92 ± 17</td>
</tr>
<tr>
<td>pH₄</td>
<td>7.31 ± 0.09</td>
<td>7.31 ± 0.08</td>
<td>7.33 ± 0.03</td>
<td>7.26 ± 0.05</td>
<td>7.29 ± 0.08</td>
</tr>
<tr>
<td>P₈CO₂ (mmHg)</td>
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<td>39 ± 6</td>
<td>39 ± 3</td>
<td>44 ± 5</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>P₈O₂ (mmHg)</td>
<td>165 ± 22</td>
<td>168 ± 23</td>
<td>186 ± 28</td>
<td>193 ± 16</td>
<td>179 ± 36</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

a total of 35 ml. The aspirated gas was then introduced into a 5350 Agent Monitor (Ohmeda, Louisville, CO) for analysis. The monitor was calibrated with known standards.

After completion of MAC determination, rats in the 1- and 24-h groups were killed by halothane overdose. For rats in the 7-day group, delivered halothane was increased to 3%. The brains were perfused via the ascending aorta with a 1 min flush of 0.9% saline followed by 250 ml of buffered 4% formalin (pH 7.35). The brains were stabilized at 4°C in situ overnight before removal and storage in 4% formalin. The brains were cut coronally into 3.0-mm-thick slices and dehydrated in graded strengths of ethanol. After rinsing in xylene and embedding in paraffin, 5-μm-thick sections were serially cut and stained with celestine blue and acid fuchsine. Brain injury was quantified by applying a damage scale to selected histologic structures (0 = no histologic changes; 1 = 1–5% of neurons damaged; 2 = 6–50% of neurons damaged; and 3: >50% of neurons damaged) by one experimenter, blind to group membership. Damage to CA1 neurons of the hippocampal formation was graded in both hemispheres at bregma −3.3 and −3.8 mm. Damage in the motor cortex (hindlimb and forelimb regions), cingulate cortex, and dorsolateral caudoputamen was graded at bregma −0.30 mm. Damage in the ventral posterior and parafascicular nuclei of the thalamus was evaluated at bregma −3.8 mm.

Anatomic regions were taken as those defined by Paxinos and Watson. Physiologic values were examined qualitatively to preserve statistical power to detect a MAC difference between the ischemic and sham-operated groups. Values for halothane MAC were compared between groups by two-way analysis of variance with independent variables as follows: (1) time between reperfusion and MAC determination, (2) ischemic or sham, and (3) interactions between the two independent variables. The statistical distribution of residuals was consistent with being normal. There were no outliers. Power analysis on values from MAC determination was performed according to methods described by Cohen. All continuous data were summarized as means ± standard deviation.

Results

Physiologic values for the experimental groups obtained before and immediately after the ischemic episode are presented in table 1. Mean arterial pressure, arterial O₂ tension, arterial CO₂ tension, arterial pH, plasma glucose and hematocrit were similar between groups before ischemia. All rats undergoing ischemia developed sustained electroencephalographic isoelectricity within 20 s of onset of ischemia. Recovery of slow-wave activity was observed within the first few minutes after reperfusion. In sham-operated animals,
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no changes in the electroencephalogram were observed during the corresponding interval. Ten min after reperfusion, arterial O₂ tension, arterial CO₂ tension, and arterial pH were similar between groups. Mean values for mean arterial pressure were 30–40 mmHg greater in the ischemic rats as compared with shams.

Rats in the 24-h and 7-day (ischemia and sham) groups recovered the righting reflex as well as grooming and feeding behavior during the respective recovery intervals. No convulsions were observed.

Halothane MAC values determined at 1 h, 24 h, or 7 days after ischemia (or sham operation) are given in figure 1. Neither ischemia (P = 0.30) nor duration of reperfusion (P = 0.30) affected MAC as compared with the sham condition. With P < 0.05 considered statistically significant, there was 80% power to detect a 0.13 vol% difference for halothane between means of the sham-operated and ischemia groups.

No rats in the 7-day–recovery sham-operated group demonstrated any histologic damage. For animals subjected to ischemia and 7 days of recovery, histologic evidence of ischemia was principally located in structures known to be selectively vulnerable to ischemia. In hippocampal CA1, six of eight rats in the ischemia group exhibited >50% of neurons damaged (grade 3). The remaining 2 rats experienced an intermediate degree of injury (grade 2). An identical pattern of damage was observed in the dorsolateral caudoputamen. Damage in the hindlimb and forelimb motor cortex was less severe; all 8 rats were assigned a grade of 1. Damage in the cingulate cortex as well as the ventral posterior and parafascicular nuclei of the thalamus consisted of only rare acidophilic neurons.

Discussion

The forebrain ischemia model used in this investigation has been well characterized and allows inferences to be made regarding the severity of insult and injury experienced by the experimental animals. A combination of bilateral carotid occlusion and hypotension (mean arterial pressure 30 mmHg) results in blood flow values <5% of control. The distribution of hypoperfusion is largely limited to forebrain structures due to continuous flow through the vertebrobasilar system into more caudal structures. Corresponding to this, histologic damage is severe in forebrain structures selectively vulnerable to ischemia, whereas essentially no damage is identifiable in the hindbrain. However, histologic consequences from 10 min of severe hypotension have not been investigated in the spinal cord, although this area of study holds potential relevance to MAC determination.

Neurologically, rats recovering from 10 min of ischemia regain the righting reflex within 30 min of reperfusion but are docile. Within 24 h, hyperexcitability to audiogenic stimuli is evident. With longer recovery, motor function remains unimpaired while memory and learning tasks continue to demonstrate deficits. From these data we conclude that neurologic substrates required for motor responses normally elicited by MAC determination studies were intact in our animals at each of the three intervals of reperfusion evaluated.

Despite 10 min of severe forebrain ischemia, MAC for halothane was unaltered at three distinct intervals of cerebral reperfusion. These results are consistent with other investigations that identified volatile anesthetic requirements to be unaltered by supratentorial lesions, as defined by MAC determination in laboratory animals. In one study, Todd et al. created a cortical freeze lesion in rats. Three days later, when ipsilateral cerebral metabolic rate was predicted to be maximally depressed, MAC for halothane was determined. There were no differences between nonlesioned control animals and those with injury. Rampil et al. made a more compelling argument that MAC is independent of supratentorial injury. In that study, via aspiration, the entire precollricular forebrain was removed. Serial MAC

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determinations made over the ensuing 1–11 h revealed no difference in anesthetic requirements from rats serving as intact controls.25 The results of these studies allow the conclusion that MAC as a measure of anesthetic potency is unlikely to be influenced by cerebral structures associated with cognition. Indeed, there is preliminary evidence that the primary site of action where volatile anesthetics cause inhibition of motor response to noxious stimuli lies in the lower motor neuron distal to synaptic intervention.26

Of note, at least two studies have identified a reduction in anesthetic requirements in animals exposed to neurologic insults. In a landmark paper that identified MAC as a measure of anesthetic potency, Eger et al. found dogs to exhibit reduced MAC for halothane when made severely hypoxic.27 That work is not inconsistent with the current study in that hypoxia presents a insult to the entire nervous system, whereas the combination of carotid occlusion and hypotension is selective for the forebrain. Thus the possibility that infratentorial structures define the response to a peripheral noxious stimulus persists for both experiments. Archer et al. evaluated the response of rats with cortical freeze injury to a tail clamp during a continuous infusion of pentobarbital.28 A 28% reduction in pentobarbital requirements was observed. Those results are discrepant with our findings and may reflect differences in MAC determination techniques, mode of drug delivery (intravenous vs. inhalation), or distinctly different mechanisms of action for pentobarbital versus halothane in attenuating motor responses to a peripheral noxious stimulus.

MAC was originally defined as a technique for measuring potency of volatile anesthetic agents. In addition, MAC has become an accepted (or even expected) technique for standardizing doses of different anesthetics when relative cardiovascular, respiratory, or cerebro-protective effects are to be compared.27 However, because anesthetic agents do more than eliminate motor responses to surgical stimuli (e.g., produce unconsciousness and physiologic homeostasis),26 the absence of forebrain involvement in the MAC assay of anesthetic potency indicates that various effects of an anesthetic agent on the central nervous system may be dependent on different anatomic sites of action.

Available evidence, however, suggests that threshold concentrations for different anesthetic effects of a drug are not dissimilar and that MAC retains value in predicting such thresholds. During clinical use, doses of volatile anesthetics are not substantially different than MAC for the respective agent.30,31 Furthermore, doses of volatile agents that are necessary to nearly abolish motor responses to noxious stimuli and also eliminate awareness are within the same order of magnitude (i.e., MAC awake is ≈60% of MAC).32 This also appears to be the case for antagonism of glutamatergic neurotransmission. The dose of NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzof(F)quinoxaline), a glutamate AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole) receptor antagonist, required to cause loss of righting reflex in the rat has been demonstrated to be similar to the dose required to reduce halothane MAC by 58%.2 MAC, as an assay of anesthetic potency, would seem to retain potential to screen for new antinociceptive agents and provide a convenient method to standardize anesthetic doses when agents are to be compared for other properties. However, the ability of MAC measurements to predict other component anesthetic requirements without concomitant studies that account for cerebral effects of the agent may be limited for certain classes of compounds (e.g., opioids).

In conclusion, because glutamatergic neurotransmission has been implicated in the mechanism of nociception and in the pathogenesis of ischemic brain damage, we postulated that a severe global ischemic insult would reduce MAC for halothane. MAC, however, was unaltered at three distinct intervals after reperfusion of the brain. These results, and those obtained from other studies, indicate that the motor response elicited as part of determination of MAC is independent of forebrain structures.

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