A Focal Cryogenic Brain Lesion Does Not Reduce the Minimum Alveolar Concentration for Halothane in Rats

Michael M. Todd, M.D.,* Julie B. Weeks, B.S.,† David S. Warner, M.D.‡

Background: A focal cortical cryogenic brain injury has been reported to reduce the brain pentobarbital concentrations needed to prevent movement in response to pain in rats. This occurred despite any apparent behavioral changes in the awake animals. To determine whether this was true with other anesthetics, the authors determined the minimum alveolar concentration (MAC) for halothane in normothermic, normocarbic ventilated Sprague-Dawley rats previously subjected to a freezing injury of the parietal cortex.

Methods: Injury was produced in halothane-anesthetized rats by applying a cold (−70° C), 4-mm-diameter brass rod to the exposed dura for 5 or 15 s. Animals then were studied 3 days after injury, a time when cerebral metabolism in the ipsilateral hemisphere reaches a minimum. Minimum alveolar concentration was determined using a tail-clamp stimulus, combined with end-tidal anesthetic sampling. In addition, exploratory activity was measured by the open field test just before MAC determination, and spontaneous nocturnal motility was monitored by an electronic motion sensor during the night before testing.

Results: In normal animals subjected only to preparatory surgery, MAC was 1.10 ± 0.07% (mean ± SD). Almost identical values were found in rats subjected to 5- and 15-s cryogenic injuries (1.11 ± 0.07% and 1.08 ± 0.06%, respectively). There were no intergroup differences in open field test results or in spontaneous nocturnal activity.

Conclusions: These results indicate that a focal cortical brain injury that has no obvious neurologic or behavioral effects in the awake rat does not alter halothane requirements. (Key words: Anesthetics, volatile: halothane. Brain injury. Potency: minimum alveolar concentration.)

IN 1991, Archer et al. reported that a standardized brain injury in rats, which was produced by freezing a portion of the cerebral cortex, reduced the cerebral tissue concentration of pentobarbital needed to render the animal unresponsive to a painful stimulus, even though the animals appeared to be neurologically normal at the time of testing.1 Injury was associated with an increase in brain tissue serotonin concentration, and both the changes in serotonin concentration and the alteration in pentobarbital requirements could be prevented by treatment with p-chlorophenylalanine, a serotonin synthesis inhibitor. Despite the care with which this study was conducted, its results may be inconsistent with the work of others. For example, Roizen et al. demonstrated that selective, raphe nucleus lesions reduced halothane minimum alveolar concentration (MAC) and cerebral serotonin concentration.2 In addition, Mueller et al. noted that treatment with p-chlorophenylalanine reduced halothane MAC in rats.3 Hence, it is possible that the changes in brain serotonin concentrations are not directly related to the observed changes in anesthetic requirements, and it is also possible that observed changes in anesthetic requirements apply to pentobarbital only and may not be generalizable to other agents. Support for both possibilities can be found. For example, when Archer et al. treated normal rats with p-chlorophenylalanine, the observed pentobarbital requirements did not differ from untreated animals. In addition, Shapira et al. noted that a percussion injury in rats did not change the inspired concentration of halothane needed to prevent movement after tail-clamping, at least as long as the animals appeared to be neurologically normal before testing.4

We set out to examine the second of these possibilities, i.e., the influence of brain injury on anesthetic requirements. Because a change in anesthetic requirements in otherwise functionally normal individuals might have both clinical and mechanistic implications, we performed two behavioral tests on rats subjected to a cryogenic brain lesion and then determined halothane MAC using the method of White et al.5

* Professor of Anesthesia and Vice Chairman for Research.
† Research Assistant II.
‡ Associate Professor of Anesthesia.

Received from the Neuroanesthesia Research Group, Dept of Anesthesiology, University of Iowa College of Medicine, Iowa City, Iowa. Accepted for publication March 19, 1993. Supported by USPHS grants RO1-NS824517 (MMT) and R29-GM39771 (DSW).

Address reprint requests to Dr. Todd: Department of Anesthesia, University of Iowa Hospitals and Clinics, GH60E, Iowa City, Iowa 52242.
Materials and Methods

The described study was reviewed and approved by the University of Iowa Animal Care and Use Committee.

Male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 375–450 g, were placed in a closed plastic box and anesthetized with oxygen and halothane. After ≈5 min, each rat’s trachea was intubated with a 14-G, 5.7-cm intravenous catheter (Jelco, Critikow, Tampa, FL). Inspired halothane concentration then was reduced to approximately 1–1.5%, delivered in 40% O₂ and nitrogen. Animals lungs were ventilated thereafter with a tidal volume of 3.2–3.5 ml, at a rate of 55 breaths/min. Rectal temperature was maintained between 37.5° and 38.0° C.

When anesthetic conditions were stable, animals were turned prone with the head supported. A midline scalp incision was made in all animals. After hemostasis was achieved, the scalp was reflected and the calvarium exposed. A 5-mm-diameter craniectomy was drilled over the left side of the skull, with the anterior margin located ≈1 mm posterior to the coronal suture and the medial margin located ≈1 mm from the sagittal suture. Animals then were assigned to one of three experimental groups:

Group 1 (control, n = 7): No further interventions. The scalp was carefully closed, halothane discontinued, and the animal awakened. After tracheal extubation, the rat remained in a warmed, oxygen-enriched atmosphere for 45 min and then was returned to the animal care unit. Free access to food and water was permitted at all times.

Group 2 (5-s cryogenic lesion, n = 7): A cryogenic lesion was created by gently placing the 4-mm-diameter tip of a cooled brass rod against the dura for 5 s. This rod was located on the end of a brass funnel, which was filled with dry ice and acetone (temperature −70° C).² After brain freezing, the rod/funnel was removed, dural bleeding was controlled with Avitene (MedChem, Woburn, MA), and the scalp was closed. Animals were awakened as in group 1.

Group 3 (15-s cryogenic lesion, n = 4): These animals were treated identically to those in group 2, except that the rod was held in contact with the dura for 15 s.

Behavioral Testing

To determine whether the lesion had any intrinsic sedative effects in the absence of anesthetics, two types of behavioral testing were performed. First, we examined spontaneous motor activity, a parameter that has been used to evaluate the pharmacodynamics of sedative drugs.⁵,⁷ This was done with an Auto-Mex II activity monitor (Columbus Instruments, Columbus, OH), which uses an electromagnetic field to quantitate movement of the animal within a metal-roofed plastic cage placed on top of the monitoring platform. On the evening before MAC determinations (i.e., the evening of the 2nd day following lesioning), animals were placed in the monitoring cages, in a darkened room at ≈5 PM, and activity was monitored for an 11-h period, between 7 PM and 6 AM the next day. Activity was summed and printed every 15 min. All movements did not yield an equivalent signal, with simple walking resulting in fewer “counts” than rearing or drinking. Therefore, we chose to report total activity counts recorded during the 11-h observation period and the percentage of the total number of 15-min epochs during which the activity count exceeded 50 (a unitless, but reproducible number). Observation indicated that epochs during which the activity count was less than 50 represented a quiescent period during which the animal was either asleep or moving very little and never feeding or drinking. Hence, an activity value of 52% would indicate that an animal was awake, active, and exploring the cage (or feeding) for 23 of the 44 possible 15-min epochs.

In addition to this measure of spontaneous motor behavior, we studied exploratory behavior using an open field test.⁹,¹⁰ This was done on the morning of the 3rd day after lesion, just before MAC determinations (after completion of spontaneous activity testing). After acclimation to a darkened room, the animals were placed in a large 3 × 3-ft open-topped box, the floor of which was divided into 36 small squares by white painted lines (on a black surface). When the animal was placed into the box, it promptly began to explore this environment, and the number of squares that the animal entered in 3 consecutive min was recorded by an observer unaware of the experimental group.

MAC Determinations

Three days after the above preparation, animals were weighed and again anesthetized with halothane and tracheally intubated, and their lungs were ventilated. Temperature was controlled, and the inspired concentration of halothane initially was adjusted to ≈1.4% (in an oxygen/nitrogen gas mixture). After 20–30 min, with the inspired halothane concentration still at 1.4%, the tail was clamped approximately 2 mm from the
CRYOGENIC BRAIN INJURY DOES NOT ALTER HALOTHANE MAC

base, using a 25-cm rubber-shod hemostat. This was wagged back and forth for 1 min, or until the animal responded with some purposeful movement, such as kicking its legs, twisting its body, or trying to roll over. Just before clamping, alveolar halothane concentration was determined by drawing gas into a glass syringe at end-expiration from a PE40 catheter, the tip of which was positioned inside the distal end of the endotracheal tube. Approximately 1 ml of gas was aspirated at the end of each of ≈50 breaths. The gas was delivered from the syringe to a Datex Model 222 volatile agent analyzer. If the animal moved in response to the tail clamp, the halothane concentration was increased another 0.15% and allowed to equilibrate for 20 min, and the tail-clamping was repeated. Otherwise, the anesthetic concentration was decreased in 0.15% increments until the animal moved. A 20-min equilibration period was allowed at each step. Once the animal responded in a purposeful fashion, the inspired anesthetic concentration was increased to a value halfway between the last two values. The end-tidal halothane concentrations closest to the "move" and "no-move" doses were averaged to determine MAC, with a resolution of ±0.07%. After MAC was determined, a blood sample was drawn percutaneously from the left ventricle of the heart for the determination of arterial oxygen tension, arterial carbon dioxide tension, and pH, and the animal was killed with an injection of potassium chloride.

Statistics
All parameters were compared using a one-way analysis of variance. All results are presented as mean ± SD.

Results
Results are summarized in table 1. Halothane MAC in control animals was 1.10 ± 0.07% (mean ± SD), and values in the 5- and 15-s freeze groups were essentially identical. There were no intergroup differences in arterial blood gases measured upon completion of the MAC determinations, nor were there any intergroup differences in weight gained or lost during the 3 days after lesion.

Spontaneous nocturnal activity and exploratory behavior did not differ between groups.

Discussion
This report describes the apparent lack of effect of a cryogenic lesion on halothane MAC as determined using standard methods. Two other measures of spontaneous activities were not influenced by the lesion.

This experiment was undertaken in response to the report by Archer et al. that a cryogenic lesion would reduce the brain concentration of pentobarbital needed to abolish all response to a painful stimulus (tail-clamping). Those authors used a 5-s cryogenic injury. Response testing was carried out 3 days later, at a time when previous studies have shown that cerebral metabolic rates for glucose ipsilateral to the lesion have reached their minimum values.12–14 Pentobarbital was infused continuously in spontaneously breathing animals, while responsiveness was tested intermittently. The endpoint was a loss of all response to a tail clamp, at which time the animals were killed. To eliminate any confusion that might arise from any blood-brain

Table 1. Experimental Results

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>5-s Freeze (n = 7)</th>
<th>15-s Freeze (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane MAC (%)</td>
<td>1.10 ± 0.07</td>
<td>1.11 ± 0.07</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>126 ± 41</td>
<td>142 ± 40</td>
<td>103 ± 28</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>36 ± 4</td>
<td>35 ± 2</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>pH (units)</td>
<td>7.49 ± 0.04</td>
<td>7.48 ± 0.04</td>
<td>7.48 ± 0.01</td>
</tr>
<tr>
<td>Weight change* (g)</td>
<td>−3.0 ± 8.8</td>
<td>4.9 ± 7.2</td>
<td>5.5 ± 5.9</td>
</tr>
<tr>
<td>Open field test (squares entered in 3 min)</td>
<td>131 ± 32</td>
<td>131 ± 29</td>
<td>121 ± 17</td>
</tr>
<tr>
<td>Spontaneous activity</td>
<td>n = 5</td>
<td>n = 6</td>
<td>n = 3</td>
</tr>
<tr>
<td>Total activity counts in 11 h</td>
<td>28,904 ± 4,188</td>
<td>32,741 ± 8,039</td>
<td>22,867 ± 3,715</td>
</tr>
<tr>
<td>% time active</td>
<td>39 ± 6</td>
<td>43 ± 6</td>
<td>37 ± 6</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
MAC = minimum alveolar concentration; PaO₂ = arterial oxygen tension; PaCO₂ = arterial carbon dioxide tension.
* Weight change is the difference between animal weight recorded before lesion and again 3 days later. Blood gas and pH values were obtained immediately after MAC determination.

Anesthesiology, V 79, No 1, Jul 1993
differences in pentobarbital concentrations, brain concentrations were measured directly.

It is difficult to find fault with the study of Archer et al. While the authors determined an ED₉₀ endpoint, which is atypical for drug-potency studies, the major limitation of such an approach would be a relative insensitivity to intervention. Since the ED₉₀ (MAC) lies at the midpoint of the sigmoid dose-response curve, it generally is considered more sensitive to change. However, Archer et al. detected a 28% reduction in pentobarbital requirements; hence, the method had adequate sensitivity. Archer et al. also measured brain rather than blood pentobarbital concentrations. Although this approach is unusual, the anesthetic effects of a drug should be related to its concentration in at least some region of the brain, and tissue concentrations clearly provide a closer estimate of this than blood levels. Only the difficulties of measuring brain tissue anesthetic concentrations have led to the use of alveolar or arterial drug concentrations to assess anesthetic actions. Serious errors can be encountered if inadequate alveolar/blood/brain equilibration times are not permitted such that arterial concentrations fail to reflect brain concentrations. Archer et al. measured the response to pain only intermittently (every 10 min) during pentobarbital infusion, rather than under steady-state conditions. It is possible that they overlooked the actual no-move point and therefore overestimated the required anesthetic doses. However, the problems with this should be limited by the use of direct brain concentrations to assess drug effect, and this would not explain the observed intergroup differences.

Instead of trying to duplicate Archer et al.'s work, we chose to carry out a more traditional MAC study to test the underlying hypothesis, largely because of the enormous body of work available using this approach and other literature concerning the influence of certain brain lesions and drugs on the measurement. Minimum alveolar concentration was determined in rats using the methods described by White et al., with the exception that gas was sampled from the distal rather than proximal end of the endotracheal tube. To standardize our approach, we approached MAC from "above," since preliminary studies indicated that this reduced variability. Hypoxia and/or hyper/hypocapnia were avoided, and normothermia was maintained at all times. This approach yielded MAC values in all of our experimental groups that are essentially identical to that reported by other authors. Other work in our laboratory has shown that we can detect relatively small changes in MAC using our methods. In addition, a power analysis estimates a >99% chance of detecting a change in MAC equal to one-half of that seen by Archer et al. (i.e., 15%). We conclude that our failure to detect a change in MAC is neither methodologic nor statistical. It is also unlikely that our cryogenic lesion differed from that of Archer et al. The design for the cryogenic probe was provided by Archer, and our freeze protocol was identical. Preliminary studies in our laboratory show that the lesion leads to the expected blood-brain barrier breakdown (as shown by Evans blue dye extravasation), a small amount of left hemispheric edema (as estimated by water content measured by wet/dry weight differences), and a qualitative unilateral reduction in cerebral metabolic rates for glucose as determined by ¹⁴C-deoxyglucose autoradiography in four animals. We chose to use a minor brain lesion (as did Archer et al.). A lesion that led to marked sedation/somnolence would be expected to alter anesthetic requirements. If a nonedating lesion altered MAC, added experiments might yield some insight into the mechanisms of general anesthesia, particularly since such minor lesions are known to produce major hemispheric changes in some neurotransmitters. Nevertheless, to eliminate the possibility that we had an injury of inadequate severity, we chose to include a small group of animals with 15-s lesions. We can find no likely, technical limitations in our experiment.

We must conclude that, in the absence of an apparent error by either Archer et al. or us, there is some difference between the effects of a cryogenic lesion on the anesthetic effects of halothane versus pentobarbital. It is impossible to speculate at present what that difference may be, although it is likely that these drugs act via different mechanisms despite their common endpoints, i.e., anesthesia. While we are hesitant to generalize this work to humans, we would speculate that cortical brain injuries that do not alter normal levels of consciousness are unlikely to have major effects on volatile anesthetic requirements.

The authors would like to thank John Long, Ph.D., for his loan of the Automex activity monitors, and Dr. John Tinker for his continued support.

References

CRYOGENIC BRAIN INJURY DOES NOT ALTER HALOTHANE MAC


