Effect of nitrous oxide on the minimum alveolar concentration for sevoflurane and the minimum alveolar concentration derivatives that prevent motor movement and autonomic responses in dogs

Reza Seddighi, DVM, PhD; Christine M. Egger, DVM, MVSc; Barton W. Rohrbach, VMD, MPH; Meredith Hobbs, DVM; Thomas J. Doherty, MVB, MSc

Objective—To investigate the effects of the concurrent administration of 70% N₂O on the minimum alveolar concentration (MAC) for sevoflurane in dogs, the MAC derivative that blocks motor movement (MAC₉₅M), and the MAC derivative that blocks autonomic responses (MAC₉₅ₐ). Animals—7 adult sexually intact male mixed-breed dogs. Procedures—For each dog, anesthesia was induced with sevoflurane delivered via a face mask. Initially, the baseline MAC, MAC₉₅M, and MAC₉₅ₐ for sevoflurane were determined by use of a noxious stimulus (50 V, 50 Hz, and 10 milliseconds) applied subcutaneously over a midulnar region. Nitrous oxide (70%) was added to the breathing circuit, and MAC, MAC₉₅M, and MAC₉₅ₐ were determined again. Percentage changes from the respective baseline concentrations for MAC, MAC₉₅M, and MAC₉₅ₐ were calculated after the administration of N₂O. Results—Baseline median values for the MAC, MAC₉₅M, and MAC₉₅ₐ for sevoflurane were 1.75%, 2.00%, and 2.50%, respectively. Addition of 70% N₂O significantly decreased MAC, MAC₉₅M, and MAC₉₅ₐ by 24.4%, 25.0%, and 35.2%, respectively, and these values did not differ significantly from each other. Conclusions and Clinical Relevance—Supplementation with 70% N₂O caused a clinically important and significant decrease in the MAC, MAC₉₅M, and MAC₉₅ₐ for sevoflurane in dogs. (Am J Vet Res 2012;73:341–345)

The potency of inhalation anesthetics is evaluated by use of the concept of MAC, which is the alveolar concentration of an anesthetic at which 50% of the population does not respond with purposeful movement to a noxious stimulus. Individual MAC values are usually determined in animal studies because the noxious stimulus may be applied repeatedly. Although the concept of MAC is widely accepted, its limitations are the subjectivity in the determination of purposeful movement and the fact that 50% of patients will move purposefully in response to surgical stimulation. Therefore, derivatives of MAC, such as MAC₉₅M and MAC₉₅ₐ, have been proposed.

Nitrous oxide is an anesthetic gas with many desirable properties, but it lacks potency in animals. The MAC for N₂O in dogs is >200% ; thus, it cannot be used as the sole anesthetic agent in this species. Nevertheless, N₂O is used as an adjunctive anesthetic, and it significantly decreases the MAC for halothane and desflurane in dogs.

The objective of the study reported here was to investigate the effects of 70% N₂O on the MAC, MAC₉₅M, and MAC₉₅ₐ for sevoflurane in dogs. We hypothesized that N₂O would significantly reduce MAC and its derivatives.

Materials and Methods

Animals—Seven healthy adult (2- to 3-year-old) sexually intact male mixed-breed dogs with a median weight of 14.5 kg (range, 11.5 to 15.0 kg) were anesthe-

ABBREVIATIONS

MAC Minimum alveolar concentration
MAC₉₅M Minimum alveolar concentration derivative that blocks autonomic responses
MAC₉₅ₐ Minimum alveolar concentration derivative that blocks motor movement
PETCO₂ End-tidal partial pressure of carbon dioxide

Received December 8, 2010. Accepted March 9, 2011.
From the Departments of Large Animal Clinical Sciences (Seddighi, Doherty), Small Animal Clinical Sciences (Egger, Hobbs), and Comparative Medicine (Rohrbach), College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996.
Address correspondence to Dr. Seddighi (mrsed@utk.edu).
tized for the study. Food was withheld for 12 hours prior to anesthesia, but access to water was allowed. The study was approved by the Institutional Animal Care and Use Committee of the University of Tennessee.

**Experimental design**—Dogs were anesthetized in random order. Each dog was anesthetized once, and MAC, MACNM, and MACBAR were determined before (baseline) and after administration of 70% N₂O.

**Anesthesia**—Anesthesia was induced with sevoflurane in oxygen delivered with a circle breathing system via a face mask. After tracheal intubation, anesthesia was maintained with sevoflurane in oxygen (2 L/min) by use of a small animal anesthesia machine. Dogs were positioned in right lateral recumbency. Ventilation was controlled to maintain the P_{ETCO₂} between 35 and 45 mm Hg. The end-tidal sevoflurane concentration, P_{ETCO₂}, and fraction of inspired oxygen were monitored continuously with an infrared gas analyzer. Samples were collected from the proximal end of the endotracheal tube at a rate of 150 mL/min. The gas analyzer was calibrated at the start of each experiment with the calibration gases (60% N₂O and a mixture of 1% sevoflurane in 5% CO₂) supplied by the manufacturer. Estimated hemoglobin saturation was monitored continuously by use of a tongue probe attached to a monitor. A standard wire 20-gauge (5.0-cm) catheter was placed in the right cephalic vein for infusion of a polyionic electrolyte solution (3 mL/kg/h). Heart rate and rhythm were monitored continuously with an ECG monitor. Arterial blood pressure was monitored continuously via a standard wire 22-gauge (2.5-cm) catheter in a dorsal pedal artery by use of a disposable transducer attached to a monitor. The midstream was selected as the zero point when dogs were in lateral recumbency. Body temperature was monitored by use of an esophageal probe, and a circulating warm water blanket and warm air blanket were used to maintain body temperature within the reference range (37.5° to 38.5°C).

**Noxious stimulus**—An electrical stimulus (50 V, 50 Hz, and 10 milliseconds) was delivered via two 25-gauge electrode needles inserted SC, 5 cm apart, over a midulnar region. Two single stimuli, with a 5-second interval between each stimulus, were delivered initially, followed 5 seconds later by a continuous stimulus of 5 seconds' duration, which was followed 5 seconds later by another continuous stimulus of 5 seconds' duration.

**Baseline MAC determination**—Approximately 45 minutes after induction of anesthesia, after the end-tidal sevoflurane concentration had been held constant at 2.5% for at least 15 minutes, determination of the baseline MAC was initiated. If purposeful movement was detected in response to noxious stimulation, the end-tidal sevoflurane concentration was increased by 0.1%; otherwise, it was decreased by 0.1% and, after a 15-minute equilibration period, the stimulus was applied again. Purposeful movement was defined as gross movement of the head or extremities, including jerking or twisting of the head or a running motion of the extremities. The noxious stimulus was discontinued immediately if purposeful movement was detected before the cycle was completed. Nonpurposeful movements, such as shivering, stiffening, and changes in the respiratory pattern, were ignored. For each dog, the baseline MAC was defined as the mean of the end-tidal sevoflurane concentration at which purposeful movement was and was not detected. All MAC determinations were performed in duplicate, and the mean value was reported; however, if the difference between 2 values was > 10%, a third determination was performed and the mean for the 3 values was calculated and reported.

**Baseline MACNM determination**—After baseline MAC was determined, the end-tidal sevoflurane concentration was maintained at 1.5 MAC for each dog for at least 15 minutes before the initiation of baseline MACNM determination. The baseline MACNM was determined in a manner similar to that used for determination of baseline MAC; however, the endpoint for baseline MACNM was the abolishment of all motor movements in response to the noxious stimulus. Twitching of the stimulated limb was not regarded as a positive response to the noxious stimulus. MOVEMENT OF THE HEAD AND CHEWING OR SWALLOWING WERE CONSIDERED POSITIVE RESPONSES TO THE STIMULUS. The lowest end-tidal sevoflurane concentration that abolished all movements in response to the stimulus was considered the baseline MACNM for each dog.

**Baseline MACBAR determination**—After baseline MACNM determination, the end-tidal sevoflurane concentration was maintained at twice the baseline MAC for each dog for at least 15 minutes before the initiation of baseline MACBAR determination. The baseline MACBAR was determined in a manner similar to that used for determination of baseline MAC and MACNM. The baseline MACBAR was defined as the minimum end-tidal sevoflurane concentration that prevented a ≥ 15% increase in mean arterial blood pressure and heart rate (from prestimulation values) in response to the noxious stimulus during a 60-second period beginning with the delivery of the first stimulus.

**Determination of MAC, MACNM, and MACBAR after N₂O treatment**—Following determination of baseline MAC, MACNM, and MACBAR, sevoflurane was delivered at each dog's baseline MAC for 15 minutes before initiating administration of 70% N₂O. Thirty minutes after achieving equilibration at an end-tidal N₂O concentration of 70%, MAC, MACNM, and MACBAR were determined again as described.

**Statistical analysis**—Percentage changes in baseline MAC, MACNM, and MACBAR after addition of 70% N₂O (treatment) were calculated. A mixed-model ANOVA was used to evaluate the effect of N₂O on MAC, MACNM, and MACBAR. Dog, treatment, and MAC endpoints (MAC, MACNM, and MACBAR) were included as classification variables. Independent variables included treatment, MAC endpoint determination, time to MAC endpoint determination, and the interaction between treatment and MAC endpoint determination. Individual dog was included as a random effect in all models. When comparing > 2 levels of an independent variable, the results were adjusted according to the Tukey method. Data were transformed to satisfy the
of 70% N2O significantly decreased the MAC, MACNM, and MACBAR after administration of 70% N2O in 7 healthy adult dogs.

**Table 1**—Median (range) values for MAC, MACNM, and MACBAR for sevoflurane before (baseline) and after administration of 70% N2O and the percentage reduction in MAC, MACNM, and MACBAR after administration of 70% N2O in 7 healthy adult dogs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>After 70% N2O administration*</th>
<th>Reduction after N2O administration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC</td>
<td>1.75 (1.40–2.15)</td>
<td>1.35 (0.85–1.65)</td>
<td>24.4 (18.2–38.3)</td>
</tr>
<tr>
<td>MACNM</td>
<td>2.00 (1.50–2.80)</td>
<td>1.65 (1.10–1.80)</td>
<td>25.0 (12.5–41.1)</td>
</tr>
<tr>
<td>MACBAR</td>
<td>2.50 (1.40–3.90)</td>
<td>1.70 (1.10–2.00)</td>
<td>35.2 (21.4–48.7)</td>
</tr>
</tbody>
</table>

*Each value differed significantly (P < 0.05) from its respective baseline value.

**Table 2**—Median (range) ratios of MACNM:MAC, MACNM:MAC, and MACBAR:MACNM for sevoflurane before (baseline) and after administration of 70% N2O in 7 healthy adult dogs.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Baseline</th>
<th>After 70% N2O administration*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACNM:MAC</td>
<td>1.16 (1.07–1.64)</td>
<td>1.18 (1.03–1.30)</td>
</tr>
<tr>
<td>MACNM:MAC</td>
<td>1.27 (1.00–2.00)</td>
<td>1.26 (0.91–1.38)</td>
</tr>
<tr>
<td>MACNM:MACNM</td>
<td>1.13 (0.93–1.39)</td>
<td>1.00 (0.86–1.21)</td>
</tr>
</tbody>
</table>

*Values were not significantly (P > 0.05) different from their respective baseline values.

Median values for baseline MAC, MACNM, and MACBAR were 1.75%, 2.00%, and 2.50%, respectively (Table 1). Addition of 70% N2O resulted in a significant decrease in MAC, MACNM, and MACBAR by 24.4%, 25.0%, and 35.2%, respectively; however, there was no significant difference in the percentage reductions among MAC and its derivatives. The median MACNM was equivalent to 1.16 MAC for sevoflurane at the baseline calculation and after N2O treatment. Median MACNM was equivalent to 1.27 MAC at the baseline calculation and 1.26 MAC after N2O treatment. These ratios did not differ significantly (Table 2). Before N2O treatment, mean ± SD heart rate was 96 ± 14 beats/min and mean arterial blood pressure was 71 ± 10 mm Hg; 15 minutes after initiating N2O administration, mean heart rate was 123 ± 12 beats/min and mean arterial blood pressure was 80 ± 8 mm Hg. The fraction of inspired oxygen was >21% and estimated hemoglobin saturation was >95% at all times after initiating N2O administration. Mean arterial blood pressure was >60 mm Hg at all times during the study.

**Discussion**

In the present study, the concurrent administration of 70% N2O significantly decreased the MAC, MACNM, and MACBAR for sevoflurane from baseline values and the magnitude of the reduction did not differ significantly among MAC and its derivatives. The baseline MAC of sevoflurane (1.75%) in the present study is less than the values (2.3%12 and 2.1%13,14) reported for dogs; however, it is comparable with the baseline MAC (1.9%15 and 1.78%16) found in other studies conducted by our laboratory group. The MAC of an inhalation anesthetic can differ substantially among animals of the same species.11 Factors affecting variability in MAC include the power of the study, type of noxious stimulus used, subjectivity in interpretation of purposeful movement, differences in the anatomic site of stimulation, and differences in physiologic variables such as PaCO2, body temperature, arterial blood pressure, and age of the test subjects.11,17 Variation within the present study was minimized by use of 1 observer (RS) for all MAC, MACNM, and MACBAR determinations and by maintaining body temperature, PaCO2, and arterial blood pressure within physiologic ranges. Electrical stimulation was used in the present study because results of another study18 indicate that MAC values determined by use of electrical and mechanical stimulation are similar. Additionally, electrical stimulation has been used by other investigators to determine MAC;16,19 in dogs;18 and cats.19

The concurrent administration of 70% N2O was associated with a 24.4% decrease in baseline MAC for sevoflurane; however, there was large variability in the percentage reduction in MAC among dogs, as evidenced by the wide range of dispersion around the median (Table 1). The magnitude of MAC reduction associated with N2O administration was consistent with the MAC-sparing effect of N2O in other studies. In dogs anesthetized with halothane, MAC was reduced by 22%20 and 33%21 with the concurrent administration of 66% N2O and 75% N2O, respectively. In dogs anesthetized with desflurane, MAC was reduced by approximately 20%22 when 50% N2O was administered concurrently; however, MAC was reduced by only 16%23 when 75% N2O was administered concurrently. In a study24 of dogs undergoing ovariohysterectomy, the concurrent administration of 64% N2O reduced the required end-tidal concentration of sevoflurane by 21.4%. The MAC-reducing effects of N2O in the present study are also comparable with effects reported in other species. In horses, concurrent administration of 25% and 50% N2O reduced the MAC for halothane by 12% and 25%, respectively.22 In Dumeril monitor lizards, concurrent administration of 66% N2O reduced the MAC for sevoflurane by 24%.23 In human subjects, the effect of N2O on the MAC of sevoflurane depends on the type of noxious stimulus and the percentage of N2O administered.24,25 The median value (2.00%) for baseline MACNM in the present study is equivalent to 1.16 baseline MAC for sevoflurane, and this ratio of MACNM to MAC for sevoflurane is comparable with a value of 1.14 for the ratio of MACNM to MAC for isoflurane found in another study2 conducted by our laboratory group. In contrast, for ponies anesthetized with halothane, a comparable endpoint to MACNM was approximately 1.6 MAC.26 The higher ratio of MACNM to MAC in that study26 in ponies, compared with the ratio in the present study, may be caused by a number of factors, including differences in species, study methods, and inhalation anesthetic. The concurrent administration of 70% N2O was associated with a 25% decrease in MACNM; however, as in the case of baseline MAC, there was large variability in the percentage reduction in MACNM among dogs.
The median value (2.50%) for baseline MAC\textsubscript{BAR} in the present study corresponds to 1.27 MAC and is similar to an MAC\textsubscript{BAR} value of 2.7% found in another study\textsuperscript{10} conducted by our laboratory group. The MAC\textsubscript{BAR} values reported from various studies differ, although in some studies, MAC and MAC\textsubscript{BAR} values are almost identical. For example, in rats anesthetized with sevoflurane, MAC\textsubscript{BAR} did not differ significantly from MAC.\textsuperscript{4} Similarly, the MAC\textsubscript{BAR} for isoflurane in cats was only 1.1 MAC.\textsuperscript{19} In contrast, the MAC\textsubscript{BAR} for halothane in cats was 1.5 MAC.\textsuperscript{27} Differences in study design, species, age of animals, and inhalation agents may account for the variability in MAC\textsubscript{BAR} results, and different inhalation agents have fundamentally different effects on MAC and its derivatives.\textsuperscript{28} For instance, the MAC\textsubscript{BAR} for desflurane and isoflurane in human patients is approximately 1.3 MAC,\textsuperscript{39} whereas the MAC\textsubscript{BAR} for sevoflurane in human patients is 3.5 MAC.\textsuperscript{31}

The sparing effect of N\textsubscript{2}O on the MAC for sevoflurane may be attributable to its angesic or immobilizing properties; however, it appears that the immobilizing actions of N\textsubscript{2}O are independent of its angesic actions.\textsuperscript{31} The angesic effect of N\textsubscript{2}O is primarily mediated via N-methyl-D-aspartate-receptor antagonism,\textsuperscript{32,33} which stimulates the hypothalamus to release corticotropin-releasing factor,\textsuperscript{34} which in turn evokes inhibitory descending nociceptive transmission.\textsuperscript{33,35} Additionally, N\textsubscript{2}O increases concentrations of endogen opioid peptides such as \(\beta\)-endorphins\textsuperscript{36,37} in the brain, which results in activation of neurons in the periaqueductal gray and locus coeruleus,\textsuperscript{38} which then modulates nociceptive processing in the spinal cord via supraspinal noradrenergic neurons\textsuperscript{31,39,40} and neurons that transmit or secrete \(\gamma\)-aminobutyric acid.\textsuperscript{41} Movement in response to a noxious stimulation is mainly elicited by activation of the ventral spinal locomotor networks, and it appears that N\textsubscript{2}O causes immobilization by blocking motor neurons in the ventral horn of the spinal cord.\textsuperscript{31,42}

In the present study, N\textsubscript{2}O significantly decreased the MAC and its derivatives for sevoflurane; however, several factors should be considered when N\textsubscript{2}O is used clinically. Hypoxemia is a potential adverse effect associated with the use of N\textsubscript{2}O, and it is imperative that hemoglobin saturation be monitored closely in patients when N\textsubscript{2}O is administered. The amount of reduction in MAC and its derivatives may vary substantially with the use of N\textsubscript{2}O, and a similar or greater reduction in MAC may be achieved by administration of commonly used injectable drugs. For example, lidocaine (50 \(\mu\)g/kg/min) and morphine (3.3 \(\mu\)g/kg/min) reduced the MAC for isoflurane in dogs by 29% and 48%, respectively.\textsuperscript{43}

In the study reported here, the concurrent administration of 70% N\textsubscript{2}O significantly decreased the MAC and its derivatives for sevoflurane in dogs. These results are in agreement with the clinical finding that N\textsubscript{2}O significantly reduces the end-tidal sevoflurane concentration needed to maintain a surgical plane of anesthesia in dogs.\textsuperscript{21}

\begin{itemize}
  \item a. North American Drager, Telford, Pa.
  \item b. Criticare Systems, Waukesha, Wis.
  \item c. Nellcor N-20V pulse oximeter, Nellcor, Pleasanton, Calif.
  \item d. Milacath, Mila International, Erlanger, Ky.
  \item e. Normosol-R, Abbott Laboratories, North Chicago, IL.
  \item f. Baxter Healthcare Corp, Irvine, Calif.
  \item g. Bair Hugger, Arazant, Minn.
  \item h. Grass Instrument Co, Quincy, Mass.
  \item i. PROC GLIMMIX, SAS, version 9.2, SAS Institute Inc, Cary, NC.
\end{itemize}

References

21. Duke T, Caillau SA, Tataryn JM. The effect of nitrous oxide on halothane, isoflurane and sevoflurane requirements in


