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

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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 (MACNM), and the MAC derivative that blocks autonomic responses (MACBAR).

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, MACNM, and MACBAR 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, MACNM, and MACBAR were determined again. Percentage changes from the respective baseline concentrations for MAC, MACNM, and MACBAR were calculated after the administration of N2O.

Results—Baseline median values for the MAC, MACNM, and MACBAR for sevoflurane were 1.75%, 2.00%, and 2.50%, respectively. Addition of 70% N2O significantly decreased MAC, MACNM, and MACBAR 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, MACNM, and MACBAR 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.1 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 MACNM and MACBAR, 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%.6,7 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 halothane6 and desflurane8,9 in dogs.

The objective of the study reported here was to investigate the effects of 70% N2O on the MAC, MACNM, and MACBAR 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-
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.6 Dogs were positioned in right lateral recumbency. Ventilation was controlled to maintain the PETCO2 between 35 and 45 mm Hg. The end-tidal sevoflurane concentration, PETCO2, and fraction of inspired oxygen were monitored continuously with an infrared gas analyzer.6 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.6 Estimated hemoglobin saturation was monitored continuously by use of a tongue probe4 attached to a monitor.6 A standard wire 20-gauge (5.0-cm) catheter6 was placed in the right cephalic vein for infusion of a polyionic electrolyte solution4 (3 mL/kg/h). Heart rate and rhythm were monitored continuously with an ECG monitor.6 Arterial blood pressure was monitored continuously via a standard wire 22-gauge (2.5-cm) catheter4 in a dorsal pedal artery by use of a disposable transducer4 attached to a monitor.6 The midstream was selected as the zero point when dogs were in lateral recumbency. Body temperature was monitored by use of an esophageal probe4, and a circulating warm water blanket and warm air blanket6 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)b was delivered via two 25-gauge electrode needles inserted SC, 5 cm apart, over a midunlar 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.10

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.13 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.13 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.2 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
Discussion

In the present study, the concurrent administration of 70% N₂O significantly decreased the MAC, MAC₇₀, 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%3,14) reported for dogs; however, it is comparable with the baseline MAC (1.9%13 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.14 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, MAC₇₀, and MACBAR determinations and by maintaining body temperature, PetCO₂, and arterial blood pressure within physiologic ranges. Electrical stimulation was used in the present study because results of another study10 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 MACBAR in dogs18 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%26 with the concurrent administration of 66% N₂O and 73% N₂O, respectively. In dogs anesthetized with desflurane, MAC was reduced by approximately 20%8 when 50% N₂O was administered concurrently; however, MAC was reduced by only 16%5 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 23% and 50% N₂O reduced the MAC for halothane by 12% and 23%, respectively.22

In Dumeril monitor lizards, concurrent administration of 25% and 50% 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 MAC in the present study is equivalent to 1.16 baseline MAC for sevoflurane, and this ratio of MAC₇₀ to MAC for sevoflurane is comparable with a value of 1.14 for the ratio of MAC₇₀ to MAC for isoflurane found in another study26 conducted by our laboratory group. In contrast, for ponies anesthetized with halothane, a comparable endpoint to MAC₇₀ was approximately 1.6 MAC.26 The higher ratio of MAC₇₀ 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 MAC₇₀, however, as in the case of baseline MAC, there was large variability in the percentage reduction in MAC₇₀ among dogs.

Table 1—Median (range) values for MAC, MAC₇₀, and MACBAR for sevoflurane before (baseline) and after administration of 70% N₂O and the percentage reduction in MAC, MAC₇₀, 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)a</td>
<td>1.35 (0.85–1.65)b</td>
<td>24.4 (18.2–39.3)</td>
</tr>
<tr>
<td>MAC₇₀</td>
<td>2.00 (1.50–2.80)c</td>
<td>1.65 (1.10–1.80)d</td>
<td>25.0 (12.5–41.1)</td>
</tr>
<tr>
<td>MACBAR</td>
<td>2.50 (1.40–3.90)e</td>
<td>1.70 (1.10–2.00)f</td>
<td>35.2 (21.4–48.7)</td>
</tr>
</tbody>
</table>

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

**Within a column, values with different superscript letters are significantly different.

Table 2—Median (range) ratios of MAC₇₀:MAC, MACBAR:MAC, and MACBAR:MAC₇₀ 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>
<th>Reduction after N₂O administration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC₇₀:MAC</td>
<td>1.16 (1.07–1.64)</td>
<td>1.16 (1.03–1.30)</td>
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</tr>
<tr>
<td>MACBAR:MAC</td>
<td>1.27 (1.00–2.00)</td>
<td>1.26 (0.91–1.38)</td>
<td></td>
</tr>
<tr>
<td>MACBAR:MAC₇₀</td>
<td>1.13 (0.93–1.39)</td>
<td>1.00 (0.86–1.21)</td>
<td></td>
</tr>
</tbody>
</table>

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

model assumption of normally distributed residuals. Values of P < 0.05 were considered significant.

Results

Median values for baseline MAC, MAC₇₀, and MACBAR were 1.73%, 2.00%, and 2.50%, respectively (Table 1). Addition of 70% N₂O resulted in a significant decrease in MAC, MAC₇₀, 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 MAC₇₀ was equivalent to 1.16 MAC for sevoflurane at the baseline calculation and after N₂O treatment. Median MACBAR 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.
The median value (2.50%) for baseline MACBAR in the present study corresponds to 1.27 MAC and is similar to an MACBAR value of 2.7% found in another study conducted by our laboratory group. The MACBAR values reported from various studies differ, although in some studies, MAC and MACBAR values are almost identical. For example, in rats anesthetized with sevoflurane, MACBAR did not differ significantly from MAC. Similarly, the MACBAR for isoflurane in cats was only 1.1 MAC. In contrast, the MACBAR for halothane in cats was 1.5 MAC. Differences in study design, species, age of animals, and inhalation agents may account for the variability in MACBAR results, and different inhalation agents have fundamentally different effects on MAC and its derivatives. For instance, the MACBAR for desflurane and isoflurane in human patients is approximately 1.3 MAC, whereas the MACBAR for sevoflurane in human patients is 3.5 MAC.

The sparing effect of N2O on the MAC for sevoflurane may be attributable to its analgesic or immobilizing properties; however, it appears that the immobilizing actions of N2O are independent of its analgesic actions. The analgesic effect of N2O is primarily mediated via N-methyl-d-aspartate-receptor antagonism, which stimulates the hypothalamus to release corticotropin-releasing factor, which in turn evokes inhibitory descending nociceptive transmission. Additionally, N2O increases concentrations of endogenous opioid peptides such as β-endorphins, which in the brain, which results in activation of neurons in the periaqueductal gray and locus coeruleus, which modulates nociceptive processing in the spinal cord via supraspinal noradrenergic neurons and neurons that transmit or secrete γ-aminobutyric acid. Movement in response to a noxious stimulation is mainly elicited by activation of the ventral spinal locomotor networks, and it appears that N2O causes immobilization by blocking motor neurons in the ventral horn of the spinal cord.

In the present study, N2O significantly decreased the MAC and its derivatives for sevoflurane; however, several factors should be considered when N2O is used clinically. Hypoxemia is a potential adverse effect as-...