The effect of nitrous oxide on the minimum alveolar concentration (MAC) and MAC derivatives of isoflurane in dogs

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Abstract

This study investigated the effects of 70% nitrous oxide (N_2O) on the minimum alveolar concentration (MAC) of isoflurane (ISO) that prevents purposeful movement, the MAC of ISO at which there is no motor movement (MAC_{NM}), and the MAC of ISO at which autonomic responses are blocked (MAC_{BAR}) in dogs.

Six adult, healthy, mixed-breed, intact male dogs were anesthetized with ISO delivered via mask. Baseline MAC, MAC_{NM}, and MAC_{BAR} of ISO were determined for each dog using a supra-maximal electrical stimulus (50 V, 50 Hz, 10 ms). Nitrous oxide (70%) was then administered and MAC and its derivatives (N_2O-MAC, N_2O-MAC_{NM}, and N_2O-MAC_{BAR}) were determined using the same methodology. The values for baseline MAC, MAC_{NM}, and MAC_{BAR} were 1.39 ± 0.14, 1.59 ± 0.10, and 1.72 ± 0.16, respectively. The addition of 70% N_2O decreased MAC, MAC_{NM}, and MAC_{BAR} by 32%, 15%, and 25%, respectively.

Résumé

Cette étude avait comme objectif d'évaluer chez des chiens les effets de 70 % d'oxyde nitreux (N_2O) sur la concentration alvéolaire minimum (MAC) d'isoflurane (ISO) qui empêche les mouvements volontaires, la MAC d'ISO à laquelle il n'y a pas de mouvement moteur (MAC_{NM}), et la MAC d'ISO à laquelle les réponses autonomes sont bloquées (MAC_{BAR}).

Six chiens mâles intacts adultes de race mélangée ont été anesthésiés avec de l'ISO administré via un masque. Les valeurs de base de MAC, MAC_{NM} et de MAC_{BAR} d'ISO ont été déterminées pour chaque chien à l'aide d'un stimulus électrique supra-maximal (50 V, 50 Hz, 10 ms). De l'oxyde nitreux (70 %) fut ensuite administré et la MAC et ses dérivées (N_2O-MAC, N_2O-MAC_{NM} et N_2O-MAC_{BAR}) déterminées à l'aide de la même méthodologie. Les valeurs des données de base de MAC, MAC_{NM} et MAC_{BAR} étaient respectivement 1,39 ± 0,14, 1,59 ± 0,10 et 1,72 ± 0,16. L'ajout de 70 % de N_2O a entrainé des diminutions de MAC, MAC_{NM} et MAC_{BAR} de 32 %, 15 % et 25 %, respectivement.

(Traduit par Docteur Serge Messier)

Introduction

The minimum alveolar concentration (MAC) of an inhalational anesthetic is defined as the alveolar concentration at sea level at which there is no purposeful movement in 50% of patients in response to a supra-maximal stimulus (1–3). Recent studies in dogs have investigated MAC derivatives, such as the MAC at which there is no motor movement (MAC_{NM}) (4) and the MAC at which the autonomic response to noxious stimuli is blocked (MAC_{BAR}) (5,6).

Nitrous oxide (N_2O) is a colorless, non-flammable gas that is used in humans for its analgesic, immobilizing, and anxiolytic effects (7–9). Compared with other inhalational anesthetics, however, N_2O is low in potency and is less potent in dogs than in humans. Reported MAC values for N_2O in dogs vary from 188% (2) to 222% (10) and N_2O is used primarily as an adjunct to volatile anesthetics for its MAC-decreasing properties. In a recent study, the authors found that 70% N_2O decreased the MAC, MAC_{NM}, and MAC_{BAR} of sevoflurane in dogs by 24%, 25%, and 35%, respectively (5).

The purpose of this study was to evaluate the effects of 70% N_2O on the MAC, MAC_{NM}, and MAC_{BAR} of isoflurane (ISO) in dogs. It was hypothesized that 70% N_2O would significantly decrease the MAC, MAC_{NM}, and MAC_{BAR} of ISO.

Materials and methods

Animals

Six adult (2 to 3 y of age), purpose-bred, mixed-breed, intact male dogs (14 ± 1 kg) were determined to be healthy based on physical examination. Food was withheld for 12 h before anesthesia, but access to water was allowed. Each dog was anesthetized once. The MAC, MAC_{NM}, and MAC_{BAR} were determined in that order for ISO alone (baseline) and then for ISO with 70% N_2O (treatment). This sequence of determination of MAC and its derivatives was used to expedite the process and is standard procedure in our laboratory.

The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Tennessee and was

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carried out in accordance with the Guide for the Care and Use of Experimental Animals.

Anesthesia

Anesthesia was induced with ISO (IsoFlo; Abbott Animal Health, Abbott Park, Illinois, USA) in oxygen delivered via mask from a circle breathing system. After tracheal intubation, anesthesia was maintained with ISO in oxygen (2 L/min) using a small animal anesthetic machine (North American Drager, Telford, Pennsylvania, USA). Ventilation was controlled to maintain the end-tidal carbon dioxide partial pressure ($P_E'CO_2$) at between 35 to 45 mmHg. Arterial blood samples were drawn from each subject at the time of each MAC, MAC_{NM}, and MAC_{BAR} determination to ensure that arterial carbon dioxide tension ($PaCO_2$), arterial partial pressure of oxygen (PaO_2), and acid-base status were within normal limits. Dogs were placed in lateral recumbency, a 20-ga cephalic catheter (MILA International, Erlanger, Kentucky, USA) was placed, and lactated Ringer's solution (Abbott Animal Health) was infused (3 mL/kg body weight per h).

End-tidal ISO (E'_{ISO}), end-tidal N_2O (E'_{N_2O}), and $P_E'CO_2$ were monitored continuously with an infrared gas analyzer (Criticare Systems, Waukesha, Wisconsin, USA). Samples were drawn from the proximal end of the endotracheal tube at a rate of 150 mL/min. At the beginning of the study, the monitor was calibrated with the calibration gases supplied by the manufacturer (1% ISO in 5% CO_2 and 60% N_2O; Criticare Systems). Body temperature was monitored using an esophageal probe (Criticare Systems). A circulating warm water blanket and a warm air blanket (Bair Hugger; Arizant Healthcare, Eden Prairie, Minnesota, USA) were used to maintain body temperature within the normal range (37.5°C to 38.5°C). Arterial blood pressure was monitored continuously from a 20-ga catheter placed in a dorsal pedal artery, using a monitor (Criticare Systems) and a disposable transducer (Baxter Healthcare Corporation, Deerfield, Illinois, USA). The middle of the sternum was taken as the zero point for blood pressure measurement. Heart rate and electrocardiogram (ECG) were monitored continuously using a 3-lead system and hemoglobin saturation (SpO_2) was monitored continuously using a tongue probe (Criticare Systems).

Determination of baseline MAC

The determination of baseline MAC began approximately 45 min after induction of anesthesia and with the E'_{ISO} held constant at 1.5% for at least 15 min. A supra-maximal stimulus (50 V, 50 Hz, 10 ms) was delivered (Grass Instrument Company, West Warwick, Rhode Island, USA) via two 25-ga electrode needles inserted subcutaneously 5 cm apart over the mid-ulnar area. Two single stimuli with a 5-s interval were delivered initially, followed 5 s later by a continuous stimulus of 5 s duration, which was repeated after 5 s (11). Purposeful movement was defined as gross movement of the head or extremities. Twitching of the stimulated limb, coughing, swallowing, rigidity, tail movement, or chewing were not considered purposeful movements. If purposeful movement occurred, the E'_{ISO} was increased by 0.1% or 0.2% depending on the magnitude of the response; otherwise, it was decreased by 0.1% and the stimulus was reapplied after a 15-min equilibration period. The MAC was defined as the mean of the lowest E'_{ISO} at which purposeful movement did and did not occur. All MAC values were determined in duplicate and the mean value was taken as the baseline MAC for that animal. If the difference between these values was greater than 10%, a third value was determined and the mean of these 3 values was taken as the baseline MAC for that animal.

Determination of baseline MAC_{NM}

After MAC was determined, the E'_{ISO} was maintained at 1.5% for at least 15 min before the baseline MAC_{NM} was determined using the same methodology as for MAC. The MAC_{NM} was defined as the lowest E'_{ISO} at which there was no motor movement, purposeful or non-purposeful, in response to the noxious stimulus. Twitching of the stimulated limb was not considered a positive response.

Determination of baseline MAC_{BAR}

After baseline MAC_{NM} was determined, the E'_{ISO} was maintained at 1.5% for at least 15 min before initiating baseline MAC_{BAR} determination. During each pre-stimulus period, heart rate (HR) and mean arterial pressure (MAP) values were recorded from the arterial line and were stable for at least 5 min, varying by less than 1%. The greatest HR and MAP values during this time period were taken as the baseline. The baseline MAC_{BAR} was determined using the same methodology as for MAC and MAC_{NM}. MAC_{BAR} was defined as the lowest E'_{ISO} that prevented a $\geq$ 15% increase in baseline MAP and HR in response to the noxious stimulus during the 60-s period beginning at the time of the first stimulus.

Administration of N_2O

After baseline MAC, MAC_{NM}, and MAC_{BAR} were determined, administration of 70% N_2O began. After a 15-min equilibration period with the E'_{N_2O} maintained at 70% and the E'_{ISO} at 1.5%, the treatment MAC endpoints (N_2O-MAC, N_2O-MAC_{NM}, and N_2O-MAC_{BAR}) were determined using the same methods previously described for the baseline MAC and its derivatives.

Time recording began immediately after the initial equilibration period and time to determination of MAC, MAC_{NM}, and MAC_{BAR} was cumulative. The dogs were evaluated for tissue damage, lameness, and pain for 24 h after recovery.

Statistical analysis

Percent change in MAC, MAC_{NM}, and MAC_{BAR} was calculated according to the formula:

$$(\text{treatment value} - \text{baseline value})/(\text{baseline value}) \times 100$$

A mixed-model analysis of variance (ANOVA) (PROC MIXED) was used to determine the effect of treatment on MAC, MAC_{NM}, and MAC_{BAR}. Dog was included as a random factor in the model. Dog, treatment, and endpoint were included as class variables. Independent variables included treatment, endpoint, time, and the 2-way interaction between endpoint and treatment. A second mixed-model ANOVA was used to compare the percent change in MAC among endpoints (MAC, MAC_{NM}, and MAC_{BAR}). Class variables included in the model were dog and endpoint. Endpoint was the independent variable and dog was included as a random factor in the model. A multiple range test according to the method of Tukey was used to adjust for multiple comparisons. Fit of the models was

Table I. Baseline and treatment MAC, MAC_{NM}, and MAC_{BAR} values of isoflurane and percent change in each value after adding 70% N_2O in 6 male dogs

MAC endpoint	Baseline	Treatment	% Change
MAC	1.39 ± 0.14^{a}	0.98 ± 0.14^{b}	-31.9 ± 3.3^{1}
MAC_{NM}	1.59 ± 0.10^{c}	1.37 ± 0.10^{c}	-14.9 ± 3.3^{2}
MAC_{BAR}	1.72 ± 0.16^{d}	1.31 ± 0.12^{e}	-24.9 ± 3.3^{1}

MAC — minimum alveolar concentration; MAC_{NM} — minimum alveolar concentration at which there is no motor movement; MAC_{BAR} — minimum alveolar concentration at which autonomic response is blocked.

[a,b,c,d,e] Values in the same row with different letters are significantly different.

[1,2] Values in the same column with different numbers are significantly different ($P \leq 0.05$). All values are presented as least squares mean ± standard error of the mean.

evaluated using the -2 log likelihood ratio and the fit of residuals from the model to a normal distribution. Residuals were evaluated using the test statistic of Shapiro-Wilk. Effect of treatment on percent change in MAC at each endpoint was evaluated using a paired *t*-test [PROC UNIVARIATE]. Data are expressed as least squares means (LSM) and standard error of the mean (SEM). A *P*-value of ≤ 0.05 was considered significant.

Results

The mean baseline values for MAC, MAC_{NM}, and MAC_{BAR} were 1.39%, 1.59%, and 1.72%, respectively (Table I). Administering 70% N_2O decreased these values by 32%, 15%, and 25%, respectively. Baseline MAC_{NM} was not significantly different than N_2O-MAC_{NM}. While the percent change in MAC_{BAR} was not significantly different than the percent change in MAC, the percent change in MAC_{NM} was significantly different than the percent change in MAC and MAC_{BAR} (Table I). The estimated hemoglobin saturation was > 95% and PaO_2, $PaCO_2$, and acid-base status were normal at all times before and during administration of N_2O. Recovery from anesthesia was uneventful and the dogs resumed normal activities within 2 to 3 h of recovery. The stimulated limbs appeared normal at all times.

Discussion

In this study, administering 70% N_2O decreased MAC, MAC_{NM}, and MAC_{BAR} (Table I). The baseline MAC value of 1.39% was comparable to the values reported for dogs in previous studies: 1.38% (12), 1.28% (13), and 1.34% (11). While interindividual variation in MAC values of 10% to 20% is typical (3), variation was minimized in this study by the use of only 1 observer. The MAC can also be affected by extremes of $PaCO_2$, PaO_2, body temperature, and arterial blood pressure. These variables were maintained within normal range in each patient throughout the experiment.

The addition of 70% N_2O decreased the MAC by 32%, which is comparable with the MAC-sparing effects of N_2O reported in previous studies. In halothane-anesthetized dogs, 75% N_2O decreased MAC by 34% (10) and in sevoflurane-anesthetized dogs, 70% N_2O decreased MAC by 24% (5). In a clinical study of dogs undergoing ovariohysterectomy, a 37% decrease in requirement for isoflurane was reported when 64% N_2O was included in the anesthetic protocol (14). These results are also consistent with the effects of N_2O in other species. For example, 70% N_2O decreased the MAC of isoflurane in rats by 40% (15) and 75% N_2O decreased the MAC in swine by 38% (16). In desflurane-anesthetized dogs, however, 70% N_2O decreased the MAC by only 16% (17). Differences among studies are likely due to individual variation, sample size, inhalational anesthetic, and experimental design.

The MAC_{NM} in this study was 1.59% or 1.14 MAC (Table I). This ratio of MAC_{NM}/MAC is comparable to the reported ratio of 1.16 for sevoflurane MAC_{NM}/MAC in dogs (5). These data are also in general agreement with a study of human surgical patients, which reported that the E'_{ISO} that prevented movement in 95% of the population was approximately 25% greater than the MAC (18). In contrast, a comparable endpoint in halothane-anesthetized ponies was equivalent to 1.6 MAC (19), which may reflect differences among species and inhalational anesthetics. The addition of 70% N_2O decreased MAC_{NM} by 15% (Table I), but there was wide variability among dogs. To the authors' knowledge, there are no published reports on the effect of N_2O on MAC_{NM} in dogs. In another study, the authors determined that 70% N_2O decreased sevoflurane MAC_{NM} in dogs by 25% (5).

In this study, baseline MAC_{BAR} was 1.72% or 1.24 MAC (Table I). This endpoint is typically greater than the other MAC derivatives, as autonomic responses are activated at lower stimulus levels and are more resistant to blockade than movement responses (20). Suppression of this response may be clinically relevant because autonomic activation can have deleterious effects on the patient (20–22). There is limited information on MAC_{BAR} in dogs and other veterinary species, and most MAC_{BAR} studies in humans include N_2O in the baseline anesthetic protocol, which makes it difficult to compare results. Recent studies by the authors reported MAC_{BAR} values of 1.27 MAC (5) and 1.4 MAC (6) for sevoflurane. Reported MAC_{BAR} values vary widely in other species. A study of isoflurane-anesthetized goats reported a MAC_{BAR} of 2.8 MAC (23), but in cats anesthetized with isoflurane, the MAC_{BAR} was only 1.1 MAC (24). In rats, the MAC_{BAR} for sevoflurane did not differ significantly from the MAC (25), and MAC_{BAR} values of 2.58 MAC (26) and 3.9 MAC (27) for sevoflurane have been reported in human female patients. Variations of such magnitude are likely due to the same factors as those discussed previously for MAC.

In the present study, the mean decrease in MAC_{BAR} with the addition of 70% N_2O was 25%. To the authors' knowledge, there are no published reports on the effect of 70% N_2O on the MAC_{BAR} of isoflurane in dogs. In a previous study, however, the authors found that 70% N_2O decreased the MAC_{BAR} of isoflurane by approximately 35% in dogs (5).

Decreases in MAC and its derivatives with N_2O could be due to its analgesic and/or immobilizing effects. Although the mechanisms of action of N_2O are not completely understood, its analgesic actions are likely separate from its immobilizing effects (9,28). Immobility during general anesthesia is mediated by motor neurons located in the ventral horn of the spinal cord (29,30). Interestingly, it has been shown that neurons in the ventral horn are more sensitive to the depressant effects of N_2O than are neurons in the dorsal horn

(31). The immobilizing effects of N_2O may be due to N-methyl-D-aspartate (NMDA) receptor blockade in the ventral horn (32,33), although mechanisms involving monoaminergic pathways have also been suggested (34).

Numerous mechanisms have been proposed to explain the analgesic actions of N_2O. Although still controversial, prevailing evidence supports the involvement of opioid and alpha-2 adrenergic receptors (28,35,36). Specifically, nitrous oxide induces analgesia by activating opioidergic neurons in the periaqueductal gray matter and noradrenergic neurons in the locus coeruleus. This results in modulation of nociceptive transmission at the level of the spinal cord (7,37–39).

In this study, the decrease in MAC and its variants with the addition of 70% N_2O ranged from 15% to 32%. It therefore appears that N_2O provides a clinically important reduction in MAC. The difference in the magnitude of the effect of N_2O on MAC and MAC_{NM} is surprising because they are both presumably mediated at the level of the spinal cord. This difference may be due to the small sample size and variability among subjects.

In this study, the determination of MAC and its derivatives was not randomized. Determining MAC provides a starting point for the determination of MAC_{NM} or MAC_{BAR}, as previously published studies and experience have indicated that MAC_{NM} and MAC_{BAR} are usually higher than MAC (5,23,26,27). Determining MAC and its derivatives in this order expedites the process. The current study and a previous study (5) from our laboratory demonstrated that the time to determine MAC and its derivatives has no significant effect on outcome, although this does not completely rule out an effect of order of determination.

The benefits of administering N_2O must be weighed against its potential adverse effects on patients, personnel, and the environment. The patient's oxygenation must be monitored continuously throughout the perioperative period, as hypoxemia is more likely when using N_2O. Long-term exposure to N_2O can have adverse effects on personnel, including bone marrow suppression, spontaneous abortion, teratogenicity, genotoxicity, and myelinopathies (39–41). In addition, N_2O contributes to ozone depletion in the environment (40,41).

In conclusion, adding 70% N_2O significantly decreased the MAC, MAC_{NM}, and MAC_{BAR} of isoflurane (ISO) in dogs by 32%, 15%, and 25%, respectively.

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References

1. Merkel G, Eger EI 2nd. A comparative study of halothane and halopropane anesthesia including a method for determining equipotency. Anesthesiology 1963;24:346–357.
2. Eger EI 2nd, Saidman LJ, Brandstater B. Minimum alveolar anesthetic concentration: A standard of anesthetic potency. Anesthesiology 1965;26:756–763.
3. Quasha AL, Eger EI 2nd, Tinker JH. Determination and applications of MAC. Anesthesiology 1980;53:315–334.
4. Seddighi R, Egger C, Rohrbach B, Cox S, Doherty T. The effects of midazolam on the end-tidal concentration of isoflurane necessary to prevent movement in dogs. Vet Anaesth Analg 2011;38:195–202.
5. Seddighi R, Egger CM, Rohrbach BW, Hobbs M, Doherty TJ. The effect of nitrous oxide on sevoflurane MAC and MAC derivatives in dogs. Am J Vet Res 2012;73:341–345.
6. Love L, Egger C, Rohrbach B, Cox S, Hobbs M, Doherty T. The effect of ketamine on the MAC_{BAR} of sevoflurane in dogs. Vet Anaesth Analg 2011;38:292–300.
7. Maze M, Fujinaga M. Pharmacology of nitrous oxide. Best Pract Res Clin Anaesthesiol 2001;15:339–348.
8. Emmanouil DE, Quock RM. Advances in understanding the actions of nitrous oxide. Anesth Prog 2007;54:9–18.
9. Jinks SL, Carstens E, Antognini JF. Nitrous oxide-induced analgesia does not influence nitrous oxide's immobilizing requirements. Anesth Analg 2009;109:1111–1116.
10. Steffey EP, Gillespie JR, Berry JD, Eger EI 2nd, Munson ES. Anesthetic potency (MAC) of nitrous oxide in the dog, cat, and stump-tail monkey. J Appl Physiol 1974;36:530–532.
11. Valverde A, Morey TE, Hernandez J, Davies W. Validation of several types of noxious stimuli for use in determining the minimum alveolar concentration for inhalation anesthetics in dogs and rabbits. Am J Vet Res 2003;64:957–962.
12. Yang XL, Ma HX, Yang ZB, et al. Comparison of minimum alveolar concentration between intravenous isoflurane lipid emulsion and inhaled isoflurane in dogs. Anesthesiology 2006; 104:482–487.
13. Steffey EP, Howland D Jr. Isoflurane potency in the dog and cat. Am J Vet Res 1977;38:1833–1836.
14. Duke T, Caulkett NA, Tataryn JM. The effect of nitrous oxide on halothane, isoflurane and sevoflurane requirements in ventilated dogs undergoing ovariohysterectomy. Vet Anaesth Analg 2006;33:343–350.
15. Santos M, Kuncar V, Martinez-Taboada F, Tendillo FJ. Large concentrations of nitrous oxide decrease the isoflurane minimum alveolar concentration sparing effect of morphine in the rat. Anesth Analg 2005;100:404–408.
16. Tranquilli WJ, Thurmon JC, Benson GJ. Anesthetic potency of nitrous oxide in young swine *(Sus scrofa)*. Am J Vet Res 1985; 46:58–60.
17. Nishimori CT, Nunes N, Paula DP, Rezende ML, Souza AP, Santos PSP. Effects of nitrous oxide on minimum alveolar concentration of desflurane in dogs. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2007;59:97–104.
18. de Jong RH, Eger EI 2nd. MAC expanded AD_{50} and AD_{95} values of common inhalation anesthetics in man. Anesthesiology 1975;42:384–389.
19. Doherty TJ, Geiser DR, Frazier DL. Comparison of halothane minimum alveolar concentration and minimum effective concentration in ponies. J Vet Pharmacol Ther 1997;20: 408–410.
20. Desborough JP. The stress response to trauma and surgery. Br J Anaesth 2000;85:109–117.

21. Ben-Eliyahu S, Page GG, Schleifer SJ. Stress, NK cells and cancer: Still a promissory note. Brain Behav Immun 2007;21:881–887.
22. Bartal I, Melamed R, Greenfeld K, et al. Immune perturbations in patients along the perioperative period: Alterations in cell surface markers and leukocyte subtypes before and after surgery. Brain Behav Immun 2010;24:376–386.
23. Antognini JF, Berg K. Cardiovascular responses to noxious stimuli during isoflurane anesthesia are minimally affected by anesthetic action in the brain. Anesth Analg 1995;81:843–848.
24. March PA, Muir WW 3rd. Minimum alveolar concentration measures of central nervous system activation in cats anesthetized with isoflurane. Am J Vet Res 2003;64:1528–1533.
25. Docquier MA, Lavand'homme P, Ledermann C, Collet V, De Kock M. Can determining the minimum alveolar anesthetic concentration of volatile anesthetic be used as an objective tool to assess antinociception in animals? Anesth Analg 2003;97:1033–1039.
26. Nakata Y, Goto T, Ishiguro Y, Terui K, Nimi Y, Morita S. Anesthetic doses of sevoflurane to block cardiovascular responses to incision when administered with xenon or nitrous oxide. Anesthesiology 1999;91:369–373.
27. Ura T, Higuchi H, Taoda M, Sato T. Minimum alveolar concentration of sevoflurane that blocks the adrenergic response to surgical incision in women: MAC_{BAR}. Eur J Anaesthesiol 1999; 16:176–181.
28. Koyama T, Fukuda K. Involvement of the kappa-opioid receptor in nitrous oxide-induced analgesia in mice. J Anesth 2010;24: 297–299.
29. Rampil IJ, King BS. Volatile anesthetics depress spinal motor neurons. Anesthesiology 1996;85:129–134.
30. Jinks S, Bravo M, Hayes SG. Volatile anesthetic effects on midbrain-elicited locomotion suggest that the locomotor network in the ventral spinal cord is the primary site for immobility. Anesthesiology 2008;108:1016–1024.
31. Kim J, Yao A, Atherley R, Carstens E, Jinks SL, Antognini JF. Neurons in the ventral spinal cord are more depressed by isoflurane, halothane, and propofol than are neurons in the dorsal spinal cord. Anesth Analg 2007;105:1020–1026.
32. Sonner JM, Antognini JF, Dutton RC, et al. Inhaled anesthetics and immobility: Mechanisms, mysteries, and minimum alveolar anesthetic concentration. Anesth Analg 2003;97:718–740.
33. Antognini JF, Atherley RJ, Dutton R, Laster MJ, Eger EI 2nd, Carstens E. The excitatory and inhibitory effects of nitrous oxide on spinal neuronal responses to noxious stimulation. Anesth Analg 2007;104:829–835.
34. Petrenko AB, Yamakura T, Kohno T, Sakimura K, Baba H. Reduced immobilizing properties of isoflurane and nitrous oxide in mutant mice lacking the *N*-methyl-D-aspartate receptor GluRε1 subunit are caused by the secondary effects of gene knockout. Anesth Analg 2010;110:461–465.
35. Guo TZ, Davies MF, Kingery WS, Patterson AJ, Limbird LE, Maza M. Nitrous oxide produces antinociceptive response via alpha 2_B and/or alpha 2_C adrenoceptor subtypes in mice. Anesthesiology 1999;90:470–476.
36. Sawamura S, Kingery WS, Davies MF, et al. Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of [alpha]2b adrenoceptors. J Neurosci 2000;20:9242–9251.
37. Zhang C, Davies MF, Guo TZ, Maze M. The analgesic action of nitrous oxide is dependent on the release of norepinephrine in the dorsal horn of the spinal cord. Anesthesiology 1999;91:1401–1407.
38. Fujinaga M, Maze M. Neurobiology of nitrous oxide-induced antinociceptive effects. Mol Neurobiol 2002;25:167–189.
39. Sanders RD, Weimann J, Maze M. Biologic effects of nitrous oxide: A mechanistic and toxicologic review. Anesthesiology 2008;109:707–722.
40. Myles PS, Leslie K, Silbert B, Paech MJ, Peyton P. A review of the risks and benefits of nitrous oxide in current anaesthetic practice. Anaesth Intensive Care 2004;32:165–172.
41. Ryan SM, Nielsen CJ. Global warming potential of inhaled anesthetics: Application to clinical use. Anesth Analg 2010;111:92–98.