Insufficient Anesthetic Potency of Nitrous Oxide in the Rat

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Nitrous oxide (N₂O) is frequently used for maintenance of anesthesia in research animals because of its minimal effect upon circulatory variables and the ability to rapidly alter its anesthetic concentration. However, N₂O's low anesthetic potency may result in inadequate levels of anesthesia under experimental conditions; thus, the ability of N₂O to provide adequate anesthesia during tracheal stimulation in rats, following withdrawal of halothane was evaluated. Twenty rats of similar ages and weights were anesthetized with halothane (1.44% inspired) and their tracheas orally intubated. Ten minutes prior to the conclusion of a 90-min halothane stabilization period, either 70% N₂O (n = 10) or 70% nitrogen (n = 10) was added to the inspired anesthetic gas mixture. The halothane was discontinued at the conclusion of the 90-min period and each rat was observed for spontaneous motor behavior while end-expiratory anesthetic gases were intermittently measured with mass spectrometry. The halothane concentrations present at the times of four specific, consistently occurring behaviors were measured, and comparisons were made between the N₂O and nitrogen groups by a mean t test. There were no significant differences (P < 0.05) in the halothane concentration between the N₂O and nitrogen groups in three of the four behaviors compared: 1) change in respiratory pattern, 2) purposeful movement of the torso, and 3) purposeful self-extubation. In the fourth behavior (purposeful movement of an extremity) the halothane concentration was higher in the N₂O group than in the nitrogen group. A mean halothane concentration of 0.49% was required to prevent the purposeful behaviors in the presence of N₂O. Thus, under conditions of this study, when utilized with sub-MAC concentrations of halothane there is no difference between the anesthetic potency of N₂O and nitrogen. When N₂O (70%) is utilized with halothane concentrations of less than 0.50% during tracheal stimulation in the rat, insufficient anesthesia is present. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: halothane. Potency: rat.)

NITROUS OXIDE (N₂O) is frequently used to provide a light level of maintenance anesthesia in animal research. It is employed with the intent of providing anesthesia with minimal physiologic perturbations. A commonly employed protocol includes induction of anesthesia with a potent anesthetic (e.g., halothane), use of muscle relaxants, followed by discontinuation of the volatile anesthetic. Subsequently, only N₂O (50–80%), with or without wound infiltration, is used to maintain anesthesia during the study period. Due to its limited potency (MAC = 136% in the rat), inadequate anesthesia may be produced when N₂O is used as the sole anesthetizing agent. Administration of N₂O under certain conditions that may prevail in laboratories may not adhere to humane considerations, as well as possibly introduce physiologic stress responses that could alter experimental data. The purpose of this study was to evaluate the anesthetic potency of N₂O in the rat, during minimally invasive noxious stimulation (orotracheal intubation), under circumstances that often occur during experiments using rodents.

Methods

With prior approval by the Institutional Animal Studies Subcommittee, male Sprague-Dawley rats (n = 20) of similar ages and weights were anesthetized in a 2.5-l plexiglass box with a fresh gas flow of 1.5 l/min consisting of a 50:50 ratio of oxygen and N₂O and 5% halothane. The rats were orotracheally intubated using the otoscope method. Briefly described, an otoscope was mounted on a ringstand with a number 2 ear speculum, the anesthetized rat was held in an erect position, and the speculum was placed in the oropharynx of the rat as the vocal cords were directly visualized. Utilizing the Seldinger technique a 20-g soft guidewire was passed into the trachea. The rat was then placed in a supine position on the surgical table and the endotracheal tube (PE-240) was passed over the guidewire into the trachea without resistance. The guidewire was removed and the endotracheal tube attached to the respiratory apparatus. This entire procedure was accomplished in 10–15 s. The position of the endotracheal tube was verified by visualization of chest expansion and by bilateral auscultation of the lung fields, and by verification of end-tidal CO₂ with mass spectrometry. Following tracheal intubation the N₂O was discontinued and a 1.44% inspired halothane concentration was administered for an equilibrium period of 90 min. This halothane level (approximately 1.3 MAC) was chosen to provide an anesthetic state that was relatively stress free. This study took place in an environment wherein no attempt to control sounds was made (ventilator noise and regular conversation) similar to many research laboratories and operating rooms. Positive pressure ventilation was maintained by a Harvard Rodent ventilator at a rate

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of 60 cycles/min and a tidal volume of 10 ml/kg of body weight. In addition to halothane, the inspired gases consisted of an air/oxygen mixture with an inspired oxygen concentration of 45%. The animals were unrestrained, and the endotracheal tube was not sutured or tied in place. Temperature was monitored rectally and was servo-controlled at 37° C.

The endotracheal tube was made of PE-240 (approximately 14-G) tubing and was attached to inspiratory and expiratory circuits via a 3-way y-connector. A rubber plug with a leak free orifice was permanently placed at the central port of the 3-way y-connector. Sampling of end-tidal gases (halothane, N₂O, nitrogen, and carbon dioxide) was achieved by passing a lubricated 20-G blunt needle through the plugged orifice of the central port to the distal tip of the endotracheal tube. Respiratory gases were analyzed, via the needle, on line by a Perkin-Elmer mass spectrometer with a sampling rate of 60 ml/min. During sampling of end-tidal gases the respiratory frequency was decreased to 40 breaths/min, and the tidal volume was increased by 33%. Preliminary studies in anesthetized rats with arterial catheters indicated that this respiratory change was necessary during mass spectrometer sampling to provide the minute ventilation necessary to maintain stable arterial blood gases, while providing a maximal end-expiratory phase for optimal mass spectrometer sampling. Anesthetic and respiratory gases were sampled at an inspiratory site, a mixed expiratory site, and an end-expiratory site. The inspiratory and mixed expiratory sites were sampled continuously, while the end-expiratory site was sampled at 10-min intervals during the 90-min halothane equilibrium period, and following halothane discontinuation at 5-min intervals until completion of the study.

After 80 min of halothane administration, each rat was randomly assigned into one of two groups. One group (n = 10) received 70% N₂O in O₂, while the second group (n = 10) received 70% nitrogen in O₂. After 10 min of either N₂O or nitrogen the halothane was discontinued. Each rat was then continuously observed for all spontaneous behavior by an investigator blinded to the constituents of the anesthetic gases. The end-point of the study was the time at which the animal's spontaneous (purposeful) activity resulted in extubation of the trachea. Although the endotracheal tube was not sutured or tied in place, the endotracheal tube was designed to fit tightly into the trachea and oropharynx such that minor movement would not dislodge it. Extubation could occur if the rat had sustained vigorous movement in a direction opposite from the respiratory apparatus, which was defined as purposeful self-extubation.

Specific behaviors that occurred in 80% or more of rats in each group during halothane elimination included the following:

1. A change in the respiratory pattern/rate—although each rat was mechanically ventilated, often the rat would increase its own spontaneous rate, or present a jerky, asynchronous pattern, as opposed to the regular pattern of breathing superimposed by the ventilator.
2. Purposeful movement of an extremity—defined as sustained movement of an extremity.
3. Purposeful movement of the torso—defined as rolling over on side, arching, or other movement of the back.
4. Purposeful self-extubation—vigorously moving in an opposite direction from a previously tolerated endotracheal tube, resulting in extubation.

Each specific behavior was evaluated between groups as to the end-tidal halothane concentration at the time of the behavior. The halothane concentration at the time of a specific behavior was interpolated from each rat's individual halothane elimination curve. Group comparisons were made between the N₂O and nitrogen group halothane concentrations for each consistently occurring (80% or greater) behavior by a mean t-test. A P value less than 0.05 was considered significant.

**Results**

While each of the four behaviors that were compared did not occur in every rat; the behaviors that were observed in each rat always occurred in the order that they are listed in table 1 (e.g., purposeful movement of an extremity always occurred following a change in respiratory pattern, and preceding purposeful movement of the torso). There was no significant difference between the N₂O and nitrogen group halothane elimination curves. Therefore, the data were combined into one curve for illustrative purposes (fig. 1).

The halothane concentration at the time of each specifically occurring behavior (occurred in at least eight
rats in each group) is listed in table 1. The time span for each rat from halothane discontinuation to the first consistently occurring behavior was always greater than 9 min. As shown in table 1, there was no statistically significant difference between groups in the end-tidal halothane concentration in the consistently occurring behaviors 1, 3, and 4 listed above. There was a difference in the halothane concentration for behavior 2 listed above. The halothane concentration was higher for the N2O group compared with that for the nitrogen group.

**Discussion**

This study demonstrates in rats, insufficient anesthetic potency of N2O (70%) in the presence of a noxious stimulus provided by an orotracheal tube. This is evident by the following observations: 1) ineffectiveness of N2O to suppress purposeful behavior when less than 0.50% halothane was present, and 2) lack of an additional anesthetic contribution by N2O as compared with nitrogen (table 1). The halothane requirement necessary to suppress purposeful movement of an extremity was greater in the N2O group. Purposeful movement of an extremity is a behavior that would be defined as a positive response if a more traditional MAC study had been done. The above interpretation of our results is limited to the conditions of this study. The present study was different from a traditional MAC study in many respects, one of which was the lack of a supramaximal stimulus. Also, the actual end-tidal halothane concentration was made at non-steady state conditions during halothane elimination; thus, the measured halothane partial pressure (end-tidal) necessary to suppress a specific behavior may have underestimated brain partial pressure due to brain tissue-alveolar halothane gradients present during elimination. In addition, while 70% nitrous oxide was an insufficient anesthetic to prevent tracheal extubation in the presence of less than 0.50% halothane, N2O may provide some contribution to the anesthetic state of the laboratory rat (analgesia, anxiolysis).9,10 Under different conditions of noxious stimuli (lesser stimuli or no stimuli at all) nitrous oxide may make enough of a contribution to the anesthetic state (analgesia and anxiolysis) as to appropriately block sensory stimuli and a stress response. Thus, it is possible that N2O may be an appropriate anesthetic for the laboratory rat under limited conditions, but not in the presence of orotracheal intubation as performed in our study.

One methodologic concern was a possible stimulatory effect provided by the end-tidal sampling technique (i.e., change in ventilation pattern and needle stimulation). This proved not to be a factor as no movement occurred during the sampling period. The sampling needle was contained within the endotracheal tube, and when sampling occurred the needle was easily passed to the tip of the endotracheal tube, without stimulating the trachea. In addition, sampling was restricted to a time period not more than 60 s. In pilot studies this time period was more than adequate for effective quantitation of the anesthetic level without evidence of a lightening of anesthesia (increase in pulse and/or blood pressure, or movement).

Beyond control of ventilation and temperature, and monitoring of inspiratory and expiratory gases, other physiologic variables were neither controlled nor monitored. This should not have accounted for the observed differences because physiologic variables within wide clinical ranges have little effect upon anesthetic requirement.11-17 In addition, the rats were separated into two groups in a random manner; as such it is likely that the actual physiologic data were commensurate between groups.

Traditionally, the potency of inhalational anesthetics are compared by MAC determinations. With this type of potency evaluation a supramaximal stimulus is administered, the response noted, and a concentration interval that contains MAC is determined.18 The present study differed in the following respects. First, less than maximal stimuli were present (endotracheal tube, a rectal thermal probe, and random auditory noise). This was done in an attempt to mimic experimental protocols that necessarily
use N₂O as the sole maintenance anesthetic in the presence of similar stimuli. In pilot studies a sub-MAC anesthetic level could be achieved that blocked responses to these stimuli, while purposeful movement was often exhibited in response to a tail clamp (supramaximal stimuli). Second, a quantitative MAC was not determined. In the present study a simple question was asked; what concentration of halothane is necessary to suppress spontaneous behavior in the presence of either N₂O or nitrogen? This question may be analogous to the MAC-awake concept previously described in humans,¹⁹,²⁰ which is defined as the minimum alveolar concentration of an anesthetic at which 50% of patients are unresponsive to commands, and are unaware of or cannot recall intraoperative events.²⁰ Obviously, it is impossible to test a rat for recall of intraoperative events. However, one can (as was done in this study) observe a rat for increasing gradations of purposeful behavior in response to stimuli. In a sense the stimuli present (endotracheal tube, rectal thermal probe, and random noise) were commands, and a response was evaluated in the context of purposeful behavior (table 1). It is hard to argue against the rat being awake at a halothane concentration of less than 0.20%. At this point the rat purposefully extubated itself, and walked away from the study field.

Although the absolute explanation for the observations of the present study requires further evaluation, three possibilities should be addressed. First is the question of acute tolerance to N₂O, which has been reported as occurring in the first 10 min of exposure to N₂O in mice,²¹ and reduces the anesthetic potency of N₂O.²² In the present study all of the behaviors that were evaluated occurred outside of a 10-min time frame. Thus, if acute N₂O tolerance were a factor, it would have been so in all of the behaviors that were evaluated. Because most research protocols that utilize N₂O as the anesthetic regimen involve administration periods far exceeding 10 min, acute N₂O tolerance should be a commensurate factor when comparing the present study to other research protocols. A second hypothesis explaining the above observations centers around the possibility that N₂O and halothane act in an antagonistic manner in selected sensory systems. Evidence supporting this hypothesis came from studies in our laboratory in which N₂O was found to activate cerebral utilization of glucose in subcortical sensory input structures.²³ This may be consistent with speculation that N₂O acts in an antagonizing manner with the volatile agent by activating a particular sensory system and making the system more receptive to sensory stimuli. In the present study multiple stimuli (e.g., endotracheal tube, rectal thermal probe, random noise) were present. Thus, the addition of N₂O may have activated one or more sensory systems, causing an increase in the transmission of sensory stimuli and a relative antagonism of the anesthetic state. However, until more is known concerning the complexity of the anesthetic mechanism of action, this remains speculative.

Finally, the possibility exists that N₂O may have increased cerebral blood flow, causing a decrease in the time necessary for halothane elimination. Because of this possibility, we did not evaluate the halothane elimination time, but the end-tidal halothane concentration at the time of a specific behavior, thus methodologically factoring out elimination time from the results. Of interest, although the two halothane elimination curves (N₂O and nitrogen) were not statistically different, there was a slight shift to the right for the N₂O halothane elimination curve as compared with the nitrogen halothane elimination curve. This indicates an increase in the time requirement for halothane elimination when N₂O was present as compared with nitrogen. Thus, this theoretical possibility was not observed in the present study.

The conclusions to be drawn from this study are that an adequate anesthetic state could not be maintained by N₂O and a halothane concentration of less than 0.50% in the presence of less than maximal stimuli in the rat. This is evident by the fact that without significant concentrations of halothane (greater than 0.49%) rats exhibited purposeful behavior. Thus, it should be expected that in experiments with either similar or more intense noxious stimuli, N₂O will not provide an adequate anesthetic state, and as a consequence the animal may be physiologically stressed. The rat may be awake as defined by the MAC-awake concept. As a corollary, when N₂O is combined with concentrations of halothane greater than 0.49%, adequate anesthesia may be present with less than maximal stimuli in the rat.

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