A Simple Device for Humidification of Inspired Gases during Volatile Anesthesia in Rats

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The typical setup for administering volatile anesthetics to laboratory rats does not provide for humidification of the inspired gases, although it is known that failing to provide humidification can lead to airway inflammation and impaired pulmonary function during prolonged experimental protocols. We developed a simple humidification system in which a nebulizer was inserted into the nonrebreathing circuit used with a standard isoflurane vaporizer. We show here that the nebulizer system resulted in anesthetic stability, as measured by withdrawal reflex latency. Although the isoflurane concentration in the delivered gases was reduced, the reduction was a consistent percentage of the vaporizer output throughout the anesthetic range, thereby permitting straightforward adjustment of the vaporizer output.

Anesthesiologists working with human patients have long recognized the importance of humidifying inspired gases, especially for procedures of prolonged duration (1). However, the potential negative consequences of inhaling dry gases are not mentioned in widely used manuals of laboratory animal anesthesia (2-4). In a series of studies in which free-breathing rats were maintained in a stereotaxic instrument using a volatile anesthetic for prolonged periods (≥ 2 h), we noted unacceptable fluctuations in anesthetic level, with massive atelectasis at the conclusion of the experiment. We suspected that these complications were due at least in part to desiccation of the air passages (5-13), as the animals were breathing gases with very low relative humidity.

To address this issue, we developed a simple humidification system in which a nebulizer was inserted into the breathing circuit used with an isoflurane vaporizer. One concern that arose with this system was the possibility that the isoflurane delivered to the animal would be reduced in an unpredictable fashion, thereby making it difficult to determine the amount of anesthetic actually delivered to the animal. Two factors could contribute to a decrease in actual percentage isoflurane. Because the humidifier was inserted downstream from the anesthesia machine common outlet, the first factor would be the addition of the water vapor to the gas flow. Second, some proportion of the isoflurane could condense within the humidifier reservoir. The first aim of the present study therefore was to determine the effect of the humidification apparatus on the concentration of isoflurane delivered to the animal. To test this, we measured isoflurane concentration in the delivered gases, with and without the humidifier in place. The second aim of the study was to document anesthetic stability in animals maintained using the humidified gases for a prolonged period (4 h). To do this, we used the latency required for the animal to remove the tail from a noxious heat stimulus (the tail flick reflex) to determine whether anesthetic depth remained stable for a period of 4 h when using the humidifier system. The logic of using a withdrawal reflex measure as a measure of anesthetic depth is that it is comparable to the movement response that is the basis for "MAC", which is the minimum alveolar concentration (MAC)of an anesthetic just sufficient to inhibit movement in 50% of patients in response to a surgical incision (14, 15). MAC is the gold-standard measure of anesthetic potency in humans (16). Other measures, including cardiovascular and respiratory variables, are considered less valuable because they are differentially affected by different anesthetics and therefore do not provide a reliable measure of anesthetic depth.

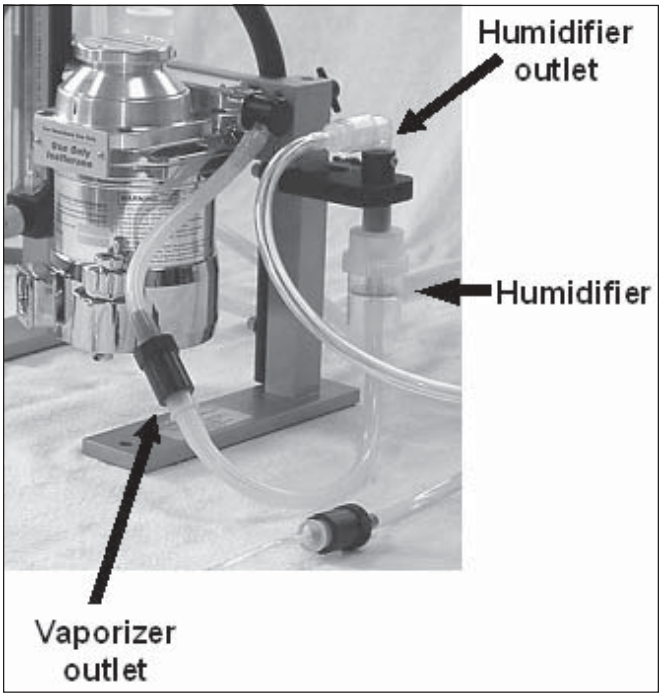

Figure 1. Humidifier device attached to a standard isoflurane vaporizer.

The humidifier was constructed using a MicroMist nebulizer (catalogue #1880, Hudson Respiratory Care Inc., Temecula, Calif.). The reservoir was filled with approximately 6 ml deionized water and mounted in a bracket (Summit Medical Equipment Co., Bend, Oreg.), which attached to the anesthesia machine (Fig. 1). Water in the nebulizer was discarded after each use and the nebulizer itself replaced on a regular basis (approximately 6 h of use). The common outlet of the vaporizer was attached to the input of the nebulizer (below the reservoir). The outlet of the nebulizer (cap end) was connected to the feed tube of a nonrebreathing system.

The effect of the humidifier on the concentration of isoflurane (Abbott Laboratories, Chicago, Ill.) in the delivered gases was first determined. A conventional isoflurane vaporizer (model 100F, Ohio Medical, Madison Wis.) was used. The flow rate was set at 1.5 liters/min in 100% O_2, and exiting gases were scavenged with no animal

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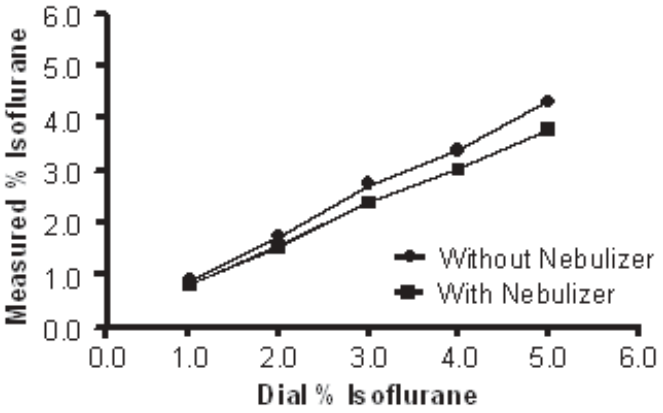

Figure 2. Delivered isoflurane remains a linear function of nominal vaporizer output following passage through the humidifier. Isoflurane concentration (mean ± SEM) in the delivered gas as a function of the vaporizer dial setting. Inserting the humidifier resulted in a loss of isoflurane amounting to approximately 10% throughout the range examined. Note that the SEMs are obscured by symbols for means. Closed circles: concentration without nebulizer, closed squares: concentration with nebulizer.

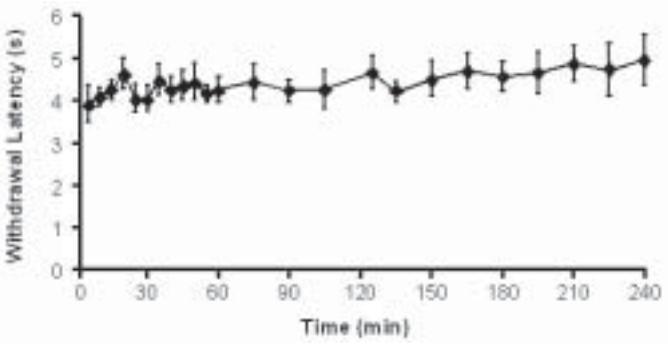

Figure 3. Humidification of inspired gases permits prolonged maintenance of rats on volatile anesthetic. Stable tail withdrawal latencies demonstrate that anesthetic state remains steady for a period of 4 h in animals maintained using the humidifier device.

in the circuit. The humidifier was filled with 6 ml deionized water. Isoflurane concentration in the delivered gases was measured after a stabilization period of at least 3 min by using a refractometer (model 1806, Riken, Tokyo, Japan). Measurements were repeated with the vaporizer dial set at 5%, 4%, 3%, 2%, and 1% with (two sets of measurements) and without (three sets of measurements) the humidifier in the circuit. Stability of the isoflurane concentration in the delivered gas over a 4-h period also was tested. The relative humidity of the delivered gases was measured using a digital thermometer–hygrometer (model 63-1013, Radio Shack, Fort Worth, Tex).

We used the latency of the tail flick reflex to noxious heat to monitor anesthetic stability over a period of 4 h in five lightly anesthetized, male, specific pathogen-free, Sprague-Dawley rats (weight, 300 to 350 g; Sasco, Wilmington, Mass.). The rats in this study were considered free from Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, H-1 virus, reovirus-3, *Mycoplasma pulmonis*, rat parvovirus, rat minute virus, CAR bacillus based on absence of antibodies to these agents in sentinel evaluation. Body temperature was maintained at approximately 37°C by a circulating water pad. All experimental procedures followed the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University. The Animal Care and Use Program at this institution is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

After chamber induction of anesthesia (4% isoflurane), animals were placed in a stereotaxic instrument (model 1220 with rat adaptor, David Kopf, Tujunga, Calif.) with a nose-cone system for reflex monitoring (initially nominally [i.e., according to the vaporizer dial] 2% isoflurane). The nose-cone system consisted of polyvinyl chloride tubing (inner diameter, 5/16 in. [ca. 7.9 mm]; outer diameter, 7/16 in. [ca. 11.1 mm]) threaded between the incisor bar and nosepiece of the stereotaxic instrument, with a 9-mm opening for insertion of the animal's nose. Animals breathed spontaneously. Once the animal was in the stereotaxic frame, isoflurane concentration was reduced in 0.25%- or 0.125%- (nominal) increments, with holding of the animal at each level for 10 min and then testing whether a tail flick reflex could be elicited (see following). Once a robust reflex with a latency of approximately 4.5 sec was obtained, the isoflurane concentration was maintained at that level for the remainder of the experiment. Reflex stability testing was started after a 30-min stabilization period. It should be emphasized that although it is possible to elicit spinal withdrawal reflexes with a noxious heat stimulus at this concentration, the animals do not move spontaneously, nor is there prolonged or excessive withdrawal to stimuli such as intense pinch. Animals were supplemented with prewarmed (approximately 42°C) lactated Ringer's solution throughout the procedure (20 ml/kg subcutaneously initially, 10 ml/kg per h thereafter).

Tail flick latency (TF) was used as a measure of anesthetic depth. Each trial consisted of a linear increase in temperature at approximately 1.8°C/sec from a holding temperature of 34°C until tail movement occurred, typically between 42 and 45°C, as previously described (17). A thermistor probe placed in contact with the skin surface of the tail provided a signal for feedback control of the heat stimulus. Trials were carried out at 5-min intervals for the first hour and at 15-min intervals for the remaining 3 h of the protocol.

Data are presented as mean ± standard error of the mean. Student's *t* test for correlated means was used to compare TF latencies during the first and last hour of stability testing; differences of $P < 0.05$ were considered significant. At the conclusion of the protocol, animals were euthanized (approximately 150 mg/kg pentobarbital intraperitoneally), and the lungs were examined for acute atelectasis.

Humidity in medical grade oxygen is typically almost zero (approximately 10 parts per million at 21°C). Including the humidifier in the output circuit resulted in a relative humidity of 34% to 35% in the delivered gases (measured over a period of 4 h). Including the humidifier in the output circuit also reduced the isoflurane concentration in the delivered gases by 10.0% ± 1.30% (mean ± SEM). This percentage reduction applied throughout the range tested (nominal output concentration, 1% through 5%; Fig. 2). Isoflurane concentration therefore remained a linear function of nominal concentration in the presence of the humidifier, and assuming the output of a particular vaporizer to be true to the dial, a dial setting of 2.00% (for example) would deliver 1.80% isoflurane to the animal. This percentage reduction also remained stable over the period of 4 h, with fluctuations of no more than 0.05% in measured isoflurane concentration over this period.

Latency of the tail flick reflex evoked by noxious heat was used as an index of anesthetic stability during a prolonged testing protocol. By using the humidifier, five animals were maintained in a lightly anesthetized state for a period of 4 h. The concentration of isoflurane in the delivered gas that was necessary to maintain this state ranged from 0.91% to 1.20% (1.04 ± 0.05%). Reflex latency remained remarkably constant throughout this period (Fig. 3), and latencies in the fourth hour were not significantly different from those in the first hour [$t(4) = 1.91$, $P = 0.12$]. In addition, necropsy showed no atelectasis in these animals.

The motivation for developing this humidifier was the need to maintain rats in a lightly anesthetized state using a volatile anesthetic for prolonged periods. When the anesthetic was delivered in dry O_2, anesthetic state varied unacceptably over time. It seemed likely that this variability was due to airway desiccation because we noted gross atelectasis (regardless of whether the nose-cone or an intubation approach was used) and because the animals often were forced to mouth-breathe when a nose-cone approach was used for a period of several hours. The constancy of the withdrawal reflex latency demonstrated here supports the stability of anesthetic state in animals maintained using this device. The absence of atelectasis is consistent with the human clinical literature supporting the importance of humidification.

As expected, passage through the humidifier reduced the isoflurane concentration reaching the animal, and this decrease may reflect the addition of water vapor to the delivered gas mix, or it may be due to condensation of isoflurane in the humidifier reservoir. However, the concentration remained a linear function of nominal (dial) concentration. Therefore, the actual concentration delivered can be approximated in a straightforward manner by reducing the nominal concentration by 10%.

This approach thus provides a simple method for humidification of inspired gases that facilitates prolonged maintenance of rats on isoflurane. One disadvantage of this approach is the possibility of increased heat loss, as the temperature of the delivered humidified gases will be reduced relative to the dry counterpart. However, this drawback can be counteracted by using warming pads and the administration of additional warmed fluids to the animal throughout the protocol.

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