

# Respiratory reflexes in response to nasal administration of halothane to anesthetized, spontaneously breathing dogs

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**Objective**—To characterize and determine the sensory innervation of respiratory reflexes elicited by nasal administration of halothane to dogs.

**Animals**—10 healthy Beagles.

**Procedure**—Dogs underwent permanent tracheostomy and, 2 to 3 weeks later, were anesthetized with thiopental and  $\alpha$ -chloralose administered IV. The nasal passages were functionally isolated so that halothane could be administered to the nasal passages while dogs were breathing 100% O<sub>2</sub> via the tracheostomy. Respiratory reflexes in response to administration of halothane at concentrations of 1.25, 1.75, and 2.5 times the minimum alveolar concentration (MAC), and 5% (administered in 100% O<sub>2</sub> at a flow rate of 5 L/min) were recorded. Reflexes in response to administration of 5% halothane were also recorded following transection of the infraorbital nerve, transection of the caudal nasal nerve, and nasal administration of lidocaine.

**Results**—Nasal administration of halothane induced an inhibition of breathing characterized by a dose-dependent increase in expiratory time and a resultant decrease in expired volume per unit time. Effects were noticeable immediately after the onset of halothane administration and lasted until its cessation. Reflex responses to halothane administration were attenuated by transection of the caudal nasal nerve and by nasal administration of lidocaine, but transection of the infraorbital nerve had no effect.

**Conclusions and Clinical Relevance**—Nasal administration of halothane at concentrations generally used for mask induction of anesthesia induces reflex inhibition of breathing. Afferent fibers in the caudal nasal nerve appear to play an important role in the reflex inhibition of breathing induced by halothane administration. (*Am J Vet Res* 2000;61:260–267)

Induction of anesthesia with volatile anesthetics is sometimes associated with development of upper airway reflexes such as apnea, breath-holding, laryngospasm, and hypersecretion,<sup>1–3</sup> as well as exaggerated

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excitement responses.<sup>2,4</sup> Such reflexes place patients at risk for exposure to hypoxia and inhibit smooth mask induction with certain volatile anesthetics, such as halothane and isoflurane, in rabbits,<sup>5,6</sup> dogs,<sup>2,4,a</sup> and humans.<sup>7,8</sup> These undesirable effects are thought to be caused by irritation of the upper airway mucosa mediated via afferent sensory nerves in the nasal passages and larynx.<sup>1,9</sup>

The larynx and nasal passage are rich in sensory afferent nerves that elicit various respiratory reflexes, such as apnea, coughing, sneezing, laryngospasm, bronchoconstriction, and mucus secretion, accompanied by sensations of touch, pain, or cooling in various species.<sup>10,11</sup> Sensory innervation of the larynx is mainly supplied by the internal branch of the cranial (superior) laryngeal nerve (CLN), and several studies have examined the effects of volatile anesthetics on the CLN. Sant'Ambrogio et al<sup>12</sup> suggested that administration of halothane to the upper airway of newborn dogs remarkably depressed ventilation, but that reflex responses to halothane disappeared following sectioning of the CLN and administration of lidocaine to the nasal passage. Laryngeal capsaicin-sensitive C-fiber receptors have been shown to be consistently activated by halothane, enflurane, and isoflurane, as determined by recording action potentials from single afferent fibers of the CLN.<sup>13</sup>

Conversely, sensory functions of the nasal passage, except for olfaction, are mediated by afferent branches of the trigeminal nerve, including the caudal (posterior) nasal nerve (CNN), infraorbital nerve (ION), and ethmoidal nerve (EN).<sup>10,11</sup> In a recent study,<sup>14</sup> the CNN was found to play a major role in reflexes elicited by application of capsaicin to the nasal mucosa of anesthetized dogs. Clinically, anesthetic induction is faster in humans when volatile anesthetics are administered through the mouth rather than through the nose, and fewer changes in breathing pattern are experienced.<sup>15</sup> In addition, Nishino et al<sup>16</sup> have shown that nasal insufflation of halothane, enflurane, or isoflurane in anesthetized humans causes changes in breathing patterns characterized by prolongation of expiration without changing tracheal smooth muscle tone.

Given these findings, it is reasonable to suppose that sensory afferent branches of the trigeminal nerve could play an important role in upper airway reflex responses to volatile anesthetics. However, little is known about the effects volatile anesthetics have on sensory afferent nerves in the nasal passages of dogs, despite the morphologic and pathophysiologic importance of the nasal passages.<sup>10,11</sup> Therefore, the purpose of the study reported

here was to determine the sensory innervation of respiratory reflexes elicited by nasal administration of halothane to dogs. We elected to use halothane in this study because it is one of the most popular halogenated volatile anesthetics in veterinary practice and is a potent stimulant of airway C-fibers in dogs.<sup>13,17,18</sup>

## Materials and Methods

**Dogs**—Ten Beagles (5 females and 5 males) were used. Mean age was 14.5 months (range, 10 to 18 months), and mean body weight was 8.6 kg (range, 7.0 to 10.5 kg). Dogs were housed in individual runs maintained at a constant temperature and humidity and fed commercial dry food once daily; water was available ad libitum. Food was withheld for 12 hours before experiments. All experimental procedures were reviewed and approved by the Animal Care Committee of the Graduate School of Agricultural and Life Sciences and performed in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences*.

**Animal preparation**—Two to 3 weeks before the study, all dogs underwent permanent tracheostomy, as described.<sup>19,20</sup> Briefly, dogs were anesthetized with sevoflurane, and placed in dorsal recumbency. An oval, midline skin incision was made, the sternohyoideus muscles were separated, and the ventral aspect of the trachea was exposed. The medial edges of the sternohyoideus muscles were sutured together dorsal to the trachea to elevate the trachea to the skin; care was taken to avoid damaging the recurrent laryngeal nerves. Ventral sections of 3 tracheal rings midway between the cricoid cartilage and the thorax were removed, leaving the tracheal mucosa intact. Mucosa was then dissected to leave an approximately 5-mm margin, and skin was sutured to the tracheal mucosa with 3-0 polypropylene monofilament suture.

Following completion of the tracheostomy, bilateral skin incisions were made at the lateral aspect of the alae nasi and extended caudally 8 to 10 cm along the nasal cavity. The zygomatic bones were carefully removed bilaterally approximately 1.5 to 2 cm caudally from the infraorbital foramen with an electric dental drill<sup>b</sup> to expose the branches of the trigeminal nerves. The bone defect was covered with absorbable gelatin sponge as necessary, and skin was sutured with 3-0 polypropylene monofilament.

Before the end of the anesthetic period, butorphanol (0.2 mg/kg of body weight) was administered IM. During the adjustment period after surgery, the tracheostomy site was carefully cleaned and nebulized with saline (0.9% NaCl) solution if necessary. Dogs were treated with cefazolin (15 mg/kg, PO, q 12 h for up to 10 days) for at least a week after surgery. Blood samples were obtained every day and submitted for CBC and serum biochemical analyses to ascertain whether the dogs were in good health.

**Experiment protocol**—For experiments in this study, dogs were sedated with a combination of an opioid and tranquilizer and anesthetized with a combination of a thiobarbiturate and  $\alpha$ -chloralose.<sup>21</sup> On the day of each experiment, dogs were premedicated with a mixture of acepromazine (0.05 mg/kg) and buprenorphine (0.01 mg/kg) administered IV through a cephalic vein catheter.<sup>22</sup> Anesthesia was induced with thiopental (1.5 to 5.0 mg/kg, IV), a cuffed tracheostomy tube<sup>c</sup> was inserted, and  $\alpha$ -chloralose (50 mg/kg, IV) was injected slowly over 15 to 20 minutes. All dogs were allowed to breathe spontaneously; 100% O<sub>2</sub> was delivered to the tracheostomy tube at a flow rate of 3 L/min, using a semi-closed circle anesthesia system.<sup>d</sup> Anesthesia was maintained by administration of  $\alpha$ -chloralose (5 mg/kg/h, IV) with an infusion pump. Adequacy of anesthesia was assessed by deter-

mining responses to tail clamping, as described,<sup>23,24</sup> 10 to 15 minutes before surgical intervention and each experimental trial, and by determining responses to pinching the interdigital skin of a hind limb every 30 minutes. If any positive response (eg, limb flinching or withdrawal or a sudden fluctuation in mean arterial blood pressure [ $> 5$  mm Hg] or heart rate [ $> 10\%$ ]) was observed, a supplemental dose of thiopental (0.5 mg/kg, IV) was given slowly, and the procedure was repeated until an adequate anesthetic depth was obtained. In pilot studies, dogs anesthetized in this manner did not have any changes in breathing patterns or blood gas partial pressures for at least 200 minutes, except that intermittent injection of thiopental sometimes caused transient apnea (most frequently characterized by prolonged expiration). Therefore, during the study, sufficient time (3 to 5 minutes) was allowed to elapse after administration of thiopental for dogs to recover a constant breathing pattern. Total cumulative doses (mean  $\pm$  SD) of thiopental and  $\alpha$ -chloralose in this study were  $5.1 \pm 1.5$  mg/kg and  $66.5 \pm 2.9$  mg/kg, respectively. Total anesthesia time was  $193 \pm 20$  minutes.

After anesthesia was induced, dogs were positioned in dorsal recumbency. Respiratory airflow was measured with a differential pressure transducer<sup>e</sup> through 2 sidearms connected to the tracheostomy tube, and integrated with an A-D converter<sup>f</sup> connected to a computer<sup>g</sup> to give tidal volume (VT). Expired volume per unit time ( $\dot{V}_E$ ) was calculated from VT and total cycle duration. A cuffed nasopharyngeal cannula (4.5 to 5.0 mm internal diameter) was introduced into the nasopharynx through the tracheostomy, and a nasal cannula with a pair of cuffed tubes was inserted into the nostrils to functionally isolate the nasal cavity (Fig 1). A polyethylene catheter (2 mm internal diameter) filled with saline solution was placed in the middle portion of the esophagus and connected to a pressure transducer<sup>h</sup> to record esophageal pressure. Inspiration time (Ti) and expiration time (TE) were measured from tracings of esophageal pressure. Respiratory frequency (fR) was calculated from Ti and TE. Arterial blood pressure was monitored with a pressure transducer<sup>h</sup> connected to a 20-gauge catheter inserted in the femoral artery. All signals were displayed on a thermal-array recorder<sup>i</sup> and recorded on magnetic tape.<sup>j</sup> Tidal gases were sampled by use of a tube connected to the tip of the tracheostomy tube, and end-tidal PCO<sub>2</sub> (PETCO<sub>2</sub>) was measured by use of an infrared gas analyzer.<sup>k</sup> Lactated Ringer's solution was infused at a rate of 10 ml/kg/h through the cephalic vein catheter. Rectal temperature was maintained at  $37 \pm 1$  C by use of a warming blanket.

**Trigeminal nerve preparation**—Small skin incisions were made along the previous surgical wounds, and the ION

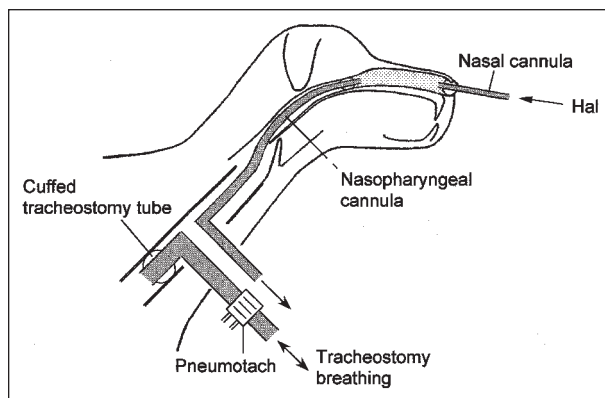


Figure 1—Schematic illustration of a method for functional isolation of the nasal passages in a dog. Halothane (Hal) is administered via the nasal cannula; the dog breathes 100% O<sub>2</sub> through the cuffed tracheostomy tube.

were isolated bilaterally at the infraorbital foramina. Next, the CNN were isolated where they emerged from the sphenopalatine foramina, using a pair of iridectomy scissors and forceps and a binocular microscope, as described.<sup>14</sup> Wounds, including the isolated nerves, were carefully covered with cotton soaked with physiologic (0.9% NaCl) solution to prevent drying.

After these experimental preparations were completed, 15 minutes was allowed to elapse so that dogs would attain a stable stage of anesthesia and cardiorespiratory function. At the commencement of each trial, PETCO<sub>2</sub> was maintained between 35 and 40 mm Hg by use of assisted manual ventilation, if necessary. In addition, if PETCO<sub>2</sub> was not within  $\pm 2$  mm Hg of the baseline value within 2 minutes after each trial, manual ventilation was also performed. For manual ventilation, frequency (approx 20 breaths/min) and peak inspiratory pressure (10 to 15 cm H<sub>2</sub>O) were increased by intermittently inflating the reservoir bag of the anesthesia system. In all dogs, manual ventilation was performed after nasal administration of 5% halothane, except when nasal anesthesia was administered and the CNN were sectioned prior to administration of 5% halothane. At least 5 minutes before each trial, animals were allowed to breathe spontaneously for 1 minute. Arterial blood samples were then collected in heparinized syringes and blood pH, PaO<sub>2</sub>, and PaCO<sub>2</sub> were measured with a blood-gas analyzer.<sup>1</sup> The next trial was performed if pH, PaO<sub>2</sub>, and PaCO<sub>2</sub> were within reference limits. Mean  $\pm$  SD values of pH, PaO<sub>2</sub>, and PaCO<sub>2</sub> during the study were  $7.37 \pm 0.07$ ,  $490 \pm 24$  mm Hg, and  $40.3 \pm 1.0$  mm Hg, respectively.

**Determination of respiratory reflexes**—Oxygen (100%) was administered through the nasal cannula at a rate of 5 L/min until respiratory cycles and intranasal temperature were constant ( $\geq 1$  minute). Halothane<sup>m</sup> was then administered through the nasal cannula at concentrations of 1.2% (1.25 times minimum alveolar concentration [MAC]<sup>24</sup>), 1.7% (1.75 MAC), 2.4% (2.5 MAC), and 5% (vaporizer set to fully open). The order of administration at each concentration was randomized, and halothane was administered at each concentration for 30 seconds, with an interval of 10 minutes before the next concentration. In 6 dogs, halothane was also administered at a concentration of 5% for up to 3 minutes to evaluate the effects of prolonged exposure.

For administration of halothane through the nasal cannula, a semi-closed circle anesthesia system<sup>a</sup> with the vaporizer<sup>n</sup> outside the circle was used. The anesthetic vaporizer that was used was specifically calibrated for use with halothane, with a vaporizer setting for each intended anesthetic concentration. Halothane concentration in the nasal

passages was measured by collecting gases from a sampling tube connected to the distal end of the nasopharyngeal cannula and measuring concentration with a gas analyzer.<sup>o</sup> Data were simultaneously downloaded to the computer,<sup>g</sup> and halothane concentration was calculated every 5 seconds.

**Determination of sensory innervation of respiratory reflexes**—After respiratory reflexes in response to nasal administration of halothane were measured, sensory innervation of those reflexes was determined. Reflex responses to administration of 5% halothane were used as the control response, because qualitatively similar respiratory reflexes were elicited by halothane at any concentration, and the greatest responses were evoked at the highest concentration.

In 4 dogs, we recorded respiratory reflexes in response to administration of 5% halothane with or without prior administration of lidocaine to the nasal passages. In the remaining 6 dogs, we recorded respiratory reflexes in response to administration of 5% halothane following sectioning of the trigeminal nerve branches. To administer lidocaine to the nasal passages, 5 ml of 2% lidocaine<sup>p</sup> was aerosolized with an ultrasonic nebulizer<sup>d</sup> driven by O<sub>2</sub> (5 L/min; output, 2.5 ml/min), producing particles approximately 5 mm in size. The aerosol was administered through the nasal cannula for 2 minutes. The nebulizer was then turned off, and respiratory reflexes in response to administration of 5% halothane were measured for 30 seconds. To determine effects of sectioning of branches of the trigeminal nerve, the ION were transected bilaterally at the infraorbital foramen, and reflexes in response to administration of 5% halothane were measured. The CNN were then transected bilaterally at the sphenopalatine foramen, and reflexes in response to administration of 5% halothane were again measured. Lidocaine was then administered to the nasal passages, and reflexes were measured a final time. In this study, the EN was left intact, because of the difficulty in isolating it without providing additional anesthesia, which would have affected baseline cardiopulmonary variables.<sup>14</sup>

At the end of the experiments, dogs were euthanized by administration of an overdose of pentobarbital (50 mg/kg, IV). Dogs were necropsied within 1 hour after death to evaluate histologic changes in the trachea caused by permanent tracheostomy. The entire trachea, including the tracheostomy site, was removed and sectioned into thin, round slices at 1-cm intervals from the tracheostomy site to the larynx. Sections were preserved in formalin, processed, and evaluated histologically. Squamous metaplasia or loss of the epithelium was observed circumferentially surrounding the tracheostomy site and extending  $3.2 \pm 0.4$  (mean  $\pm$  SD) cm

Table 1—Respiratory and cardiovascular variables associated with nasal administration of halothane at 1.25, 1.75, and 2.5 times the minimum alveolar concentration (MAC) to 10 anesthetized, spontaneously breathing dogs

Variable	1.25 MAC		1.75 MAC		2.5 MAC	
	Baseline	Peak response	Baseline	Peak response	Baseline	Peak response
Ti (s)	1.2 $\pm$ 0.1	1.3 $\pm$ 0.2	1.1 $\pm$ 0.2	1.2 $\pm$ 0.2	1.1 $\pm$ 0.3	1.2 $\pm$ 0.2
Te (s)	2.7 $\pm$ 0.4	3.0 $\pm$ 0.5	3.3 $\pm$ 0.3	4.7 $\pm$ 0.3*	3.5 $\pm$ 0.4	5.8 $\pm$ 0.5*,†
fR (breaths/min)	15 $\pm$ 2	14 $\pm$ 3	14 $\pm$ 3	11 $\pm$ 4*	14 $\pm$ 2	9 $\pm$ 4*,†
Vt (ml)	181 $\pm$ 13	178 $\pm$ 20	182 $\pm$ 16	185 $\pm$ 17	183 $\pm$ 15	178 $\pm$ 19
$\dot{V}_E$ (L/min)	2.7 $\pm$ 0.2	2.6 $\pm$ 0.3	2.6 $\pm$ 0.2	2.2 $\pm$ 0.3*	2.6 $\pm$ 0.2	1.8 $\pm$ 0.4*,†
PETCO <sub>2</sub> (mm Hg)	41 $\pm$ 2	43 $\pm$ 3	39 $\pm$ 2	45 $\pm$ 3*	42 $\pm$ 2	48 $\pm$ 4*,†
HR (beats/min)	93 $\pm$ 6	93 $\pm$ 8	95 $\pm$ 7	95 $\pm$ 7	95 $\pm$ 6	95 $\pm$ 6
MAP (mm Hg)	101 $\pm$ 8	99 $\pm$ 6	98 $\pm$ 8	99 $\pm$ 8	95 $\pm$ 5	96 $\pm$ 7

\*Significantly ( $P < 0.05$ ) different from baseline value. †Significantly ( $P < 0.05$ ) different from value obtained with administration of halothane at 1.25 MAC.  
 Ti = Inspiration time. Te = Expiration time. fR = Respiratory frequency. Vt = Tidal volume.  $\dot{V}_E$  = Expired volume per unit time.  
 PETCO<sub>2</sub> = End-tidal PCO<sub>2</sub>. HR = Heart rate. MAP = Mean arterial blood pressure.  
 Mean  $\pm$  SEM halothane concentrations were  $1.18 \pm 0.02\%$  (1.25 MAC),  $1.72 \pm 0.03\%$  (1.75 MAC), and  $2.38 \pm 0.03\%$  (2.5 MAC).  
 Data are reported as mean  $\pm$  SEM.

Table 2—Respiratory and cardiovascular variables associated with nasal administration of 5% halothane in 10 anesthetized, spontaneously breathing dogs in which the trigeminal nerves were intact and after transection of the infraorbital nerve (ION), transection of the caudal nasal nerve (CNN), and anesthesia of the nasal passages by administration of lidocaine

Variable	Nerve intact		ION section		CNN section		Nasal anesthesia	
	Baseline	Peak response	Baseline	Peak response	Baseline	Peak response	Baseline	Peak response
	Ti (s)	1.1 ± 0.2	1.3 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Te (s)	3.1 ± 0.3	7.1 ± 0.7*	3.3 ± 0.4	6.9 ± 0.7*	3.5 ± 0.3	4.2 ± 0.5*,†,‡	3.4 ± 0.3	3.4 ± 0.3†,‡,§
fR (breaths/min)	15 ± 2	7 ± 3*	13 ± 3	7 ± 3*	13 ± 2	11 ± 2*,†,‡	12 ± 2	12 ± 2†,‡,§
Vt (ml)	180 ± 13	158 ± 18*	187 ± 10	165 ± 17*	193 ± 10	188 ± 16	188 ± 11	187 ± 13†,‡
VE (L/min)	2.7 ± 0.2	1.4 ± 0.3*	2.5 ± 0.2	1.4 ± 0.3*	2.5 ± 0.2	2.2 ± 0.3*	2.5 ± 0.1	2.5 ± 0.2†,‡,§
PETCO <sub>2</sub> (mm Hg)	41 ± 2	54 ± 3*	42 ± 2	52 ± 3*	40 ± 2	45 ± 3*,†,‡	42 ± 1	43 ± 2†,‡,§
HR (beats/min)	92 ± 5	92 ± 6	90 ± 6	90 ± 6	93 ± 5	93 ± 5	95 ± 4	95 ± 6
MAP (mm Hg)	96 ± 6	100 ± 9	98 ± 7	99 ± 8	95 ± 7	96 ± 7	95 ± 7	97 ± 6

\*Significantly ( $P < 0.05$ ) different from baseline value. †Significantly ( $P < 0.05$ ) different from value obtained when nerves were intact. ‡Significantly ( $P < 0.05$ ) different from value obtained after transection of the ION. §Significantly ( $P < 0.05$ ) different from value obtained after transection of the CNN.  
See Table 1 for remainder of key.

from the site; however, no histologic changes were observed in the laryngeal mucosa.

**Data analyses**—Respiratory variables ( $T_i$ ,  $T_e$ ,  $f_R$ ,  $V_T$ ,  $\dot{V}_E$ , and  $PETCO_2$ ) were analyzed on a breath-by-breath basis. Baseline values for respiratory variables were obtained by averaging values for 3 consecutive breaths immediately before each trial. Mean arterial blood pressure (MAP) was calculated every 5 seconds as the sum of diastolic pressure plus a third of pulse pressure. Heart rate (HR) was derived electrophysiologically from the blood pressure signal. Baseline values for the cardiovascular variables were obtained by averaging values for 1 minute before each trial. Experimental values were peak responses recorded after the onset of halothane administration.

For comparisons of the differences in baseline values for each trial, one-way ANOVA was used. To determine whether the change from baseline to experimental values within each trial was significant, a paired Student *t*-test was used. Evoked changes ( $\Delta$ ) from baseline values in each trial were compared over treatments and dogs, using two-way ANOVA. When the interaction was significant, the Tukey post hoc test was used. For comparisons of evoked changes among treatments (nerves intact, following sectioning of the ION, following sectioning of the ION and CNN, and following sectioning of the ION and CNN and nasal administration of lidocaine), one-way ANOVA followed by the Tukey post hoc test was used. Values of  $P < 0.05$  were considered significant. All data were expressed as mean  $\pm$  SEM, unless otherwise indicated.

Figure 2—Changes in expiratory time ( $T_e$ ), tidal volume ( $V_T$ ), and expired volume per unit time ( $\dot{V}_E$ ) in response to nasal administration of halothane in dogs. \* = Significantly ( $P < 0.05$ ) different from baseline value. † = Significantly ( $P < 0.05$ ) different from value obtained with administration of halothane at a concentration of 1.2%. ‡ = Significantly ( $P < 0.05$ ) different from value obtained with administration of halothane at a concentration of 1.75%. § = Significantly ( $P < 0.05$ ) different from value obtained with administration of halothane at a concentration of 2.4%.

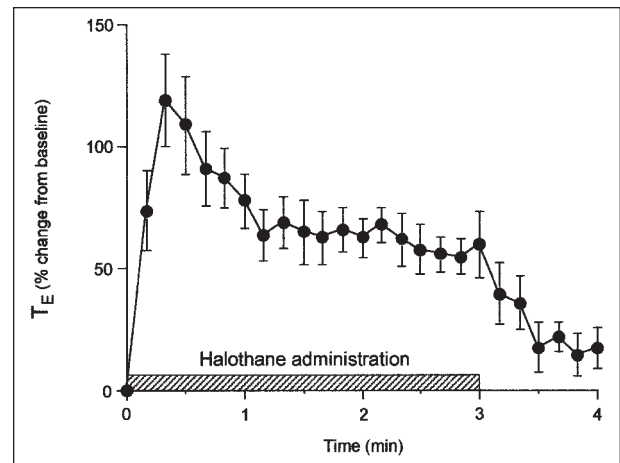
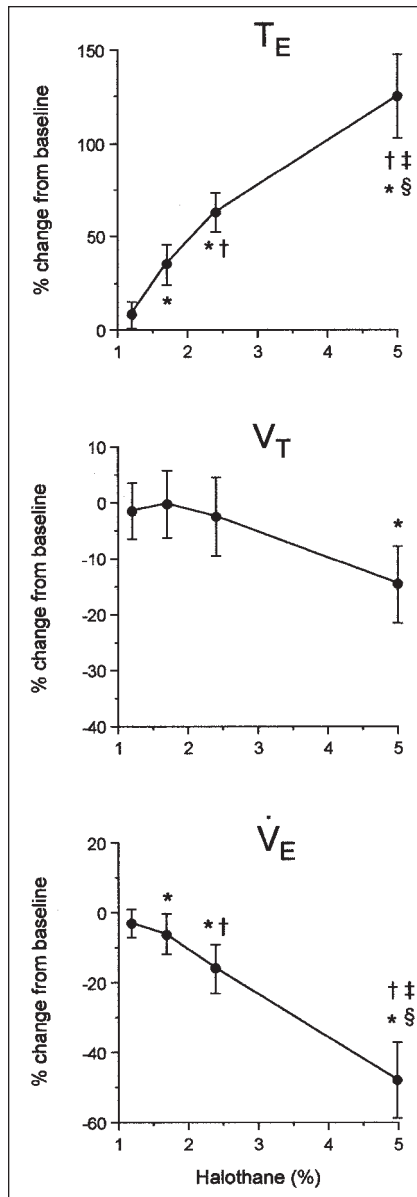


Figure 3—Effects of nasal administration of 5% halothane for 3 minutes on  $T_e$  in dogs.



## Results

**Respiratory reflexes in response to nasal administration of halothane**—Baseline values of respiratory and cardiovascular variables were not significantly different among halothane concentrations or treatments (Tables 1 and 2). Nasal administration of halothane for 30 seconds induced an inhibition of breathing characterized by a dose-dependent increase in  $T_E$  and a result-

ant decrease in  $\dot{V}_E$  (Fig 2). A significant decrease in  $V_T$  was observed in response to administration of 5% halothane. A dose-dependent increase in  $P_{ETCO_2}$  was detected and attributed to inhibition of ventilation. Peak responses were detected after  $20.2 \pm 3.4$  seconds with administration of 1.2% halothane,  $21.0 \pm 3.5$  seconds with administration of 1.7% halothane,  $18.6 \pm 3.5$  seconds with administration of 2.4% halothane, and  $16.0 \pm 2.9$  seconds with administration of 5% halothane; however, time to peak response was not significantly different among concentrations.

When 5% halothane was administered for 3 minutes,  $T_E$  began to increase immediately (Fig 3), with peak response  $15.6 \pm 2.6$  seconds after the onset of administration. Thereafter, the effect began to wane but lasted until cessation of halothane administration. Peak response and time to peak response after administration of 5% halothane for 3 minutes and administration for 30 seconds were not significantly different.

Values of  $T_i$ , HR, and MAP were not significantly changed in any trial during the study (Tables 1 and 2).

**Trigeminal nerve innervation of respiratory reflexes in response to nasal administration of halothane**—Respiratory reflexes in response to nasal administration of 5% halothane were no longer evoked in any of the 4 dogs following administration of lidocaine to the nasal passages, suggesting that the respiratory reflexes were mediated by sensory afferent nerves in the nasal mucosa.<sup>14</sup>

Respiratory reflexes in response to administration of 5% halothane were not significantly changed by bilateral transection of the ION but were significantly inhibited following bilateral transection of the CNN (Fig 4; Table 2). Mild, but significant, inhibition of breathing characterized by an increase in  $T_E$  was abolished by nasal administration of lidocaine. A typical example of respiratory reflexes in response to nasal administration of 5% halothane in which a dog in the trigeminal nerves were intact and after transection of the ION, transection of the

Figure 4—Changes in  $T_E$ ,  $V_T$ , and  $\dot{V}_E$  in response to nasal administration of halothane in dogs in which the trigeminal nerves were intact and after transection of the infraorbital nerve (ION), transection of the caudal nasal nerve (CNN), and anesthesia of the nasal passages by administration of lidocaine. \* = Significantly ( $P < 0.05$ ) different from baseline value. † = Significantly ( $P < 0.05$ ) different from value obtained when nerves were intact. ‡ = Significantly ( $P < 0.05$ ) different from value obtained after transection of the ION. § = Significantly ( $P < 0.05$ ) different from value obtained after transection of the CNN.

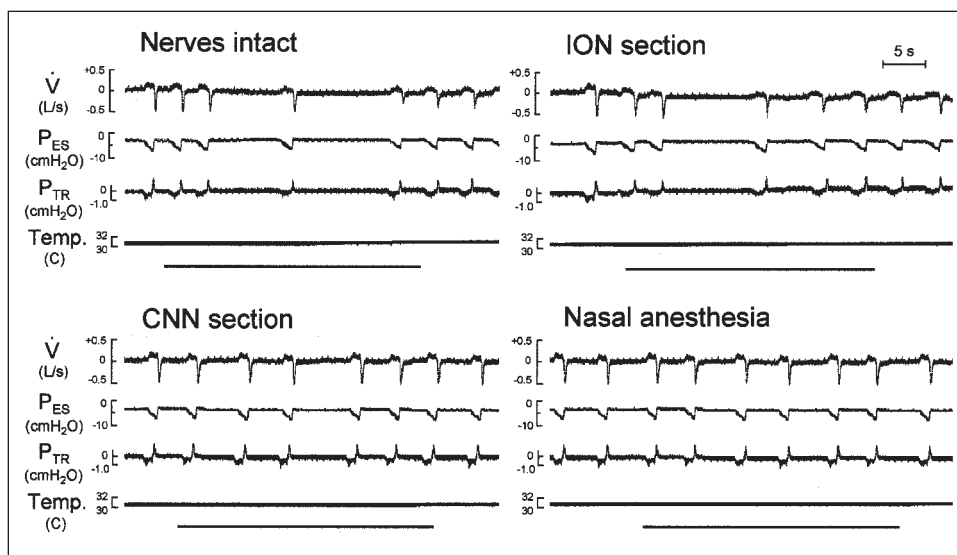
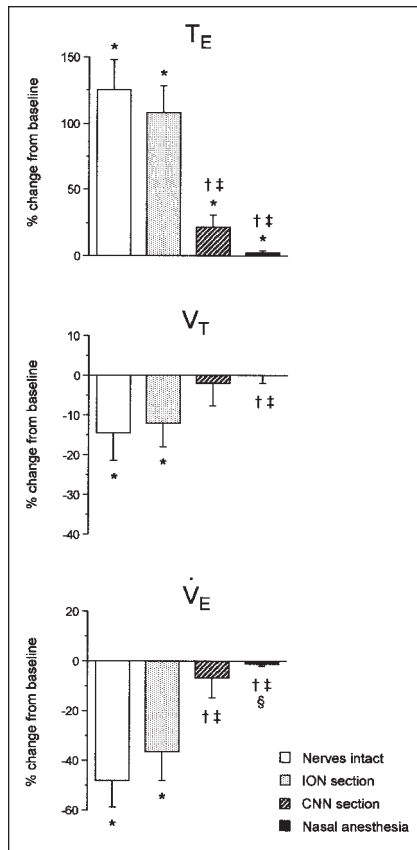


Figure 5—Experimental records illustrating respiratory reflexes in response to nasal administration of 5% halothane for 30 seconds in an anesthetized, spontaneously breathing dog.  $\dot{V}$  = Respiratory airflow.  $P_{ES}$  = Esophageal pressure.  $P_{TR}$  = Intratracheal pressure. Temp = Nasal temperature.

CNN, and anesthesia of the nasal passages by administration of lidocaine is illustrated (Fig 5).

## Discussion

Animal preparation and choice of anesthetic are key to the successful study of reflex responses of the respiratory tract.<sup>19,20,a</sup> One of the most important considerations in studies of upper airway reflexes in anesthetized animals is the amount of surgical stimulation, because surgical stimulation requires a deeper plane of anesthesia, which could mask the normal reflex pathways. In fact, approximately 2 to 3 times the dose of injectable anesthetic is generally required in experiments similar to the one described in the present report if dogs are undergoing acute tracheostomy preparation. This may partly explain the lack of reflex responses to administration of halothane in adult dogs in a previous study.<sup>12</sup> This was also why we elected to perform a permanent tracheostomy at least 2 weeks before studying reflex responses to halothane administration in the present study.

We elected to anesthetize dogs in this study with  $\alpha$ -chloralose, because  $\alpha$ -chloralose minimally depresses circulation and respiration and maintains stability of the autonomic nervous system,<sup>21</sup> making it useful for physiologic research. Because the anesthetic effect of  $\alpha$ -chloralose is enhanced when thiobarbiturates, opioids, or tranquilizers are first administered,<sup>21</sup> we chose to premedicate dogs with a tranquilizer-opioid combination and induce anesthesia with thiopental. Potential modulation of reflex responses by the background anesthetic is a problem inherent in all studies of reflex responses in anesthetized animals; however, to our knowledge, there is not any evidence that use of  $\alpha$ -chloralose as a background anesthesia masks respiratory reflex responses to volatile anesthetics.<sup>12,a</sup> Furthermore, although apnea associated with administration of low doses of thiopental (0.5 mg/kg, IV) may be expected to affect ventilatory variables for the subsequent trials, we tried to minimize this effect by allowing dogs a sufficient period to recover a normal breathing pattern. It is unclear whether changing plasma concentrations of buprenorphine and acepromazine after bolus administration may affect breathing pattern of animals. However, we believe that use of buprenorphine (0.01 mg/kg) and acepromazine (0.05 mg/kg) had little effect on results of the present study, because respiratory variables stay within reference limits following administration of these drugs at these doses to dogs.<sup>22</sup> Overall, the background anesthetic protocol used in the present study appeared to be appropriate for maintaining stable ventilatory variables for a long period.

We initially considered using sevoflurane or propofol infusion plus epidural anesthesia for background anesthesia in the present study, because of their properties in recent clinical studies.<sup>25,r</sup> However, both were discarded because they caused substantial respiratory depression when dogs were breathing spontaneously and decreased reflex responses to halothane administration in a pilot study.

Early studies of respiratory reflexes suggested that stimulation of the nasal mucosa initiated pronounced airway reflexes resulting in apnea, glottal closure,

mucus secretion, bronchoconstriction, and sneezing in many species.<sup>10,11</sup> Most of these reflexes were thought to be mediated by sensory receptors involved in the trigeminal nerves. Nasal mechanoreceptors that act in response to cooling, pressure, and water, and nasal chemoreceptors that act in response to chemical substances such as capsaicin, have been demonstrated by recording trigeminal nerve afferent action potentials in dogs, cats, rats, and guinea pigs.<sup>11,14,26,27</sup> Results of the present study suggest that the respiratory reflex responses can also be induced by administration of halothane to the nasal passages of anesthetized adult dogs. In this study, respiratory responses to administration of halothane consisted mainly of a dose-dependent increase in  $T_E$  and a decrease in  $V_T$ . Such changes are similar to those observed following administration of 5% halothane to the larynx of newborn (5 to 14 days old) dogs, in which  $\dot{V}_E$  decreased 38%, compared with baseline value, secondary to a decrease in  $f_R$  and  $V_T$ .<sup>12</sup> In that study, the effects of halothane administration were considerably reduced by transection of the CLN. In addition, results were age-dependent, with the greatest responses to halothane administration in dogs < 2 weeks old and a decrease in responses by 4 to 5 weeks of age and a lack of responses in adult dogs. Although it is still unclear how maturity affects sensitivity to halothane, taken together these findings may indicate that the reflex response to halothane administration in adult dogs originates in the nose rather than the larynx. This would correspond to the observation that in adult humans anesthetic induction is faster when sevoflurane is administered through the oral cavity, rather than the nasal passages.<sup>15</sup>

There was no doubt that respiratory reflexes in response to nasal administration of halothane in the present study were mediated by stimulation of afferent nerves in the nasal mucosa, because the effects of halothane administration were considerably reduced by local anesthesia of the nasal passages and by transection of branches of the trigeminal nerve. Because sensory innervation of the nasal mucosa and nostril is supplied by the maxillary (CNN and ION) and ophthalmic (EN) branches of the trigeminal nerve, these trigeminal nerve branches convey a variety of stimuli arising from the nose.<sup>10,11</sup> In a recent study, afferent branches of the CNN were found to have a role in reflex control of breathing in dogs.<sup>14</sup> Moreover, the sneeze reflex can be evoked by electrical stimulation of the CNN,<sup>11</sup> and intranasal administration of chemical irritants (eg, ammonia vapor, distilled water, or capsaicin) has been reported to increase the afferent activity of the CNN in dogs and cats.<sup>26,27</sup> Thus, results of the present study strongly suggest that reflex responses to halothane administration are mainly related to stimulation of afferent fibers of the CNN. Mild reflex inhibition of breathing in response to halothane administration that remained after transection of the CNN may arise from afferent fibers of the EN, because these reflexes were completely inhibited by anesthesia of the nasal passages.

The mechanism that determines what type of sensory afferent nerves are activated by nasal administration of halothane is still unclear. Capsaicin-sensitive

C-fibers located in the nasal mucosa may be involved. It is well known that apnea, coughing, sneezing, bronchoconstriction, mucus secretion, and extravasation are reflexively elicited by stimulation of airway C-fibers via a local axon or a central pathway.<sup>28,29</sup> Recently, C-fibers that respond to capsaicin, a potent stimulator of airway C-fibers, have been identified in the nose and larynx of dogs, using single nerve unit recordings of the CNN or CLN,<sup>13,27</sup> and it has been shown electrophysiologically that capsaicin-sensitive receptors were uniformly activated in a dose-dependent manner by laryngeal administration of halothane; whereas, other sensory receptors, such as respiration-modulated receptors or rapidly adapting irritant receptors, showed variable responses.<sup>13,30,31</sup> Moreover, responsiveness of capsaicin-sensitive receptors to halothane is closely associated with elicitation of reflex responses to halothane in the present study, that is, a robust peak response followed by long-lasting activity. With regard to a relationship between the C-fiber defense reflex and the nasal irritant property of halothane, Cervin and Lindberg<sup>32</sup> point out that acceleration of mucociliary activity in response to halothane administration was mediated by local axon and cholinergic central reflex mechanisms as a result of stimulation of C-fibers in the rabbit maxillary sinus in vivo, suggesting that C-fibers have an important role in reflex responses of the nasal mucosa to halothane.

Clinically, use of halothane for mask induction of anesthesia in dogs, cats, rabbits, and humans results in apnea, breath-holding, coughing, sneezing, laryngospasm, bronchoconstriction, secretion, or movement.<sup>1-9</sup> Some of these respiratory reflexes, such as apnea and breath-holding, are still present even when halothane is administered to sedated animals,<sup>6</sup> and the degree of airway irritation reportedly varies with anesthetic depth.<sup>9</sup> In the present study, we did not elicit any powerful defense reactions, such as the coughing and sneezing frequently observed in humans,<sup>1,7-9</sup> even though the highest concentration of halothane (5%) was administered, because the dogs were already anesthetized. Differences among species may account for these varied defense responses.<sup>33</sup> In clinical practice, we usually observe apnea or breath-holding, rather than coughing or sneezing, during mask induction of anesthesia with halothane in small animals such as dogs and rabbits.<sup>2,3,5,6</sup>

Theoretically, uptake of anesthetic gas by blood lowers the alveolar (end-tidal) concentration during induction with the more soluble volatile anesthetics such as halothane. For this reason, a 3 to 5% concentration of halothane is generally used for mask induction of anesthesia in dogs, cats, rabbits, and humans.<sup>2-6,34</sup> In the present study, we were able to simulate a rise in end-tidal halothane concentration by administering various concentrations of halothane, and results suggested that the nasal passages are the most vulnerable to exposure to high anesthetic concentrations. Thus, it is expected clinically that inhalation of halothane would induce a dose-dependent inhibition of breathing that in turn would delay anesthetic uptake. Such respiratory reflex responses may result in a prolonged induction period with excessive body movements and ventilatory depres-

sion during rapid mask induction.<sup>4</sup> This suggests that there may be an advantage to using conventional incremental mask induction, rather than rapid mask induction. Moreover, we consistently found a prolonged increase in PETCO<sub>2</sub> after administration of 5% halothane, which may support the suggestion that acute inhalation of high concentrations of halothane is disadvantageous. In any case, careful ventilatory monitoring is needed when halothane is used for mask induction of anesthesia.

<sup>a</sup>Mutoh T. *Studies on the actions of volatile anesthetics to the sensory system in the respiratory tract of the dog*. PhD thesis, The University of Tokyo, 1998.

<sup>b</sup>BL-F2, Osada Medical, Tokyo, Japan.

<sup>c</sup>Portex, Nihon Medical Co, Tokyo, Japan.

<sup>d</sup>Model KA-3020, Kimura Medical Co, Tokyo, Japan.

<sup>e</sup>DD102A, Toyoda Machine Works, Tokyo, Japan.

<sup>f</sup>Mac Lab Scope, BRC Inc, Tokyo, Japan.

<sup>g</sup>PowerBook 5300cs, Apple Computer Inc, Cupertino, Calif.

<sup>h</sup>DX-300, Nihon Kohden, Tokyo Japan.

<sup>i</sup>RT3100N, NEC san-ei, Tokyo, Japan.

<sup>j</sup>PC 204A, Sony Co, Tokyo, Japan.

<sup>k</sup>Respina 1H26, NEC-sanei, Tokyo, Japan.

<sup>l</sup>IL-1303, Instrumentation Laboratory Inc, Lexington, Mass.

<sup>m</sup>Halothane, Hoechst Japan Co, Tokyo, Japan.

<sup>n</sup>Fluotec Mark 3, Cyprane North America Inc, Orchard Park, NY.

<sup>o</sup>AGM-103 Capnomac, Datex, Helsinki, Finland.

<sup>p</sup>Xylocaine, Astra-Japan, Osaka, Japan.

<sup>q</sup>NE-U12, Omron Co, Tokyo, Japan.

<sup>r</sup>Branson KR, Quandt JE, Martinez EA, et al. Multi-site clinical trial of sevoflurane (abstr). *Vet Surg* 1997;26:156.

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