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Effects of Varying Concentrations of Halothane on the Activity of the Genioglossus, Intercostals, and Diaphragm in Cats: An Electromyographic Study

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To determine the possible differential effects of depth of inhalation anesthetics on inspiratory muscle activity, the following were studied in seven adult cats: the phasic activity of the diaphragm, the external intercostals, and the genioglossus, by means of electromyography (EMG) and its moving time average (MTA). The animals spontaneously breathed 1.0–3.0% halothane in O_2, while arterial P_{CO_2} was maintained constant at approximately 60 mmHg by adjusting CO_2 in the inspired gas mixture. Muscle activity was evaluated in terms of peak height of MTA, with measurements at 1% halothane used as control values. Halothane anesthesia attenuated inspiratory muscle activity significantly ($P < 0.05$) in a dose-dependent fashion; muscle activity decreased most in the genioglossus, least in the diaphragm, and intermediately in the intercostals. Respiratory frequency, inspiratory time, and inspiratory duty cycle did not change significantly with increasing concentration of halothane.(Key words: Anesthetics, volatile: halothane. Measurement technique: electromyography. Muscle, skeletal: diaphragm; external intercostals; genioglossus. Ventilation: effects of anesthetics.)

UNDER PHYSIOLOGIC conditions, ventilation is achieved by coordinated contraction of various inspiratory muscles, including the diaphragm, external intercostals, and muscles of the neck and upper airway. During general anesthesia, ventilation is almost always depressed and ventilatory pattern is often changed. The mechanism of such ventilatory depression has not been well studied. Intercostal activity is known to be more easily depressed by inhalation anesthetics, such as ether and halothane, than is the diaphragm.[1,2] Recent reports showed that the activity of the genioglossus, which plays an important role in maintaining upper airway patency during inspiration, is easily depressed by sleep, alcohol ingestion, and general anesthesia.[3–6] Such depression of upper airway muscles results in upper airway obstruction.[7] Although these findings suggest that various respiratory muscles and their motor innervation have different degrees of sensitivity to anesthetics, a direct comparison of the three major inspiratory muscle groups has not been reported. Also lacking are studies in spontaneously breathing animals without an artificial airway, mechanical ventilation, or cortical transection. The purpose of the current study was to evaluate quantitatively the effect of inhalation anesthesia (halothane) on the phasic activity of inspiratory muscles in spontaneously breathing cats without interference with the airways (endotracheal intubation) or respiratory control (mechanical ventilation, muscle relaxant, or decerebration). We compared the electromyographic activity of the diaphragm, the external intercostals, and the genioglossus in response to increasing depth of halothane anesthesia.

Materials and Methods

With the approval of the Institutional Animal Care and Use Committee, we studied seven 2-yr-old cats (mean ± SD: 2,950 ± 920 g; range, 1950–4500 g). Anesthesia was induced with 1.5–2.5% halothane in oxygen *via* a face mask; the animals breathed spontaneously throughout the experiments. Then the animal's head was placed in a lucite hood or head box. Halothane–oxygen mixture was delivered into the hood with minimal leak around the neck to provide anesthesia at the flow rate of 6 l/min to eliminate contamination or dilution by room air and rebreathing of endogenous CO_2. The hood was connected to a nonrebreathing anesthesia circuit, which consisted of one-way valves and a fully expanded 0.5–l reservoir bag under low positive pressure. Excess gas flow was drained out of the hood through a scavenging tube (fig. 1). The halothane vaporizer (Draegerwerk, Luebeck, Germany) had been calibrated with a mass spectrometer (Perkin-Elmer MGA100, Pamona, California). After the induction of anesthesia, a catheter was inserted into the left femoral artery for arterial blood gas analysis and blood pressure monitoring. Body temperature was monitored by a rectal thermistor and kept within normal limits for cats (37.0–39.0° C) using a warming blanket.

The EMG electrodes were multistrand stainless steel wires with teflon insulation (Bergen Cable Technical Co., Lodi, New Jersey). The wire was threaded through a 21-g needle, approximately 0.5 mm of insulation at the tip was scraped off with a blade, then the bare tip was bent toward the barrel of the needle. A pair of EMG electrodes

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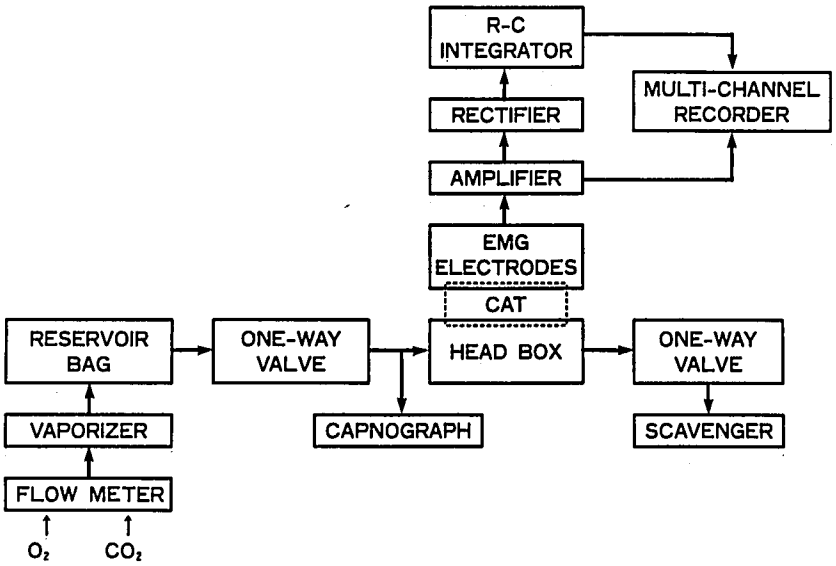

FIG. 1. The experimental setup.

was inserted into each muscle using a guided needle technique,[8] and a reference electrode was inserted into hind leg muscles. Electrodes in the diaphragm were inserted transcutaneously into the crural portion, using a technique described previously.[9]

Electrodes in the intercostal muscles were inserted into the right 5th or 6th posterior intercostal space approximately 1 mm apart using direct exposure through a skin incision just below the scapula. The incisions were subsequently closed. Electrodes in the genioglossus were inserted through the underside of the tongue. After all electrodes were inserted, the animals were turned over and kept prone. Head position was unchanged throughout the study because a change in head position alters genioglossus activity.[10] After the experiments the animals were killed by injection of pentobarbital. The position of the electrodes was confirmed at necropsy.

The raw EMG signals were amplified by a DC preamplifier (Model EMA 830, CWE, Inc., Admore, Pennsylvania) and high-impedance head-stage amplifiers (Model HSA-830, CWE, Inc.). Signals were amplified 1,000–10,000 times (noise and artifacts were filtered below 50 Hz and above 5 Hz) and recorded on an electrostatic recorder with a linear frequency response up to approximately 5 kHz (model ES 1000, Gould Inc., Cleveland, Ohio). EMG signals were also rectified and integrated (moving time average, MTA) by an RC circuit (SA-414 Analog Processor, Service Associate, Inc., San Diego, California), the time constant of which was 50 or 100 ms. We chose an appropriate time constant for the animal, according to the respiratory frequency, and kept it constant throughout the study. MTA and raw EMG signals were recorded on a multichannel recorder[11] (fig. 1). Inspiratory muscle activity was quantitated by the peak height of MTA (*i.e.*, the difference between end-expiratory baseline activity of MTA and the peak activity of MTA during inspiration).

Because our pilot studies revealed that inspired halothane concentration of 3.5% or greater in cats resulted in either apnea or extreme hypoventilation (unpublished data), subsequent experiments were conducted at inspired concentrations of 1.0–3.0% halothane. The concentration of halothane in the hood reached the inflow concentration within 30 s after a change in inflow concentration. In addition, our pilot studies in cats with an endotracheal tube showed that the difference between inspiratory and end-expiratory halothane concentrations was abolished within 5 min after a change in inflow concentration. We thus concluded that 20 min is more than sufficient time for the animal to reach a steady state.

After surgery, the concentration of halothane in O_2 was increased to and maintained at 3.0%, and arterial blood gas tensions were measured. Pa_{CO_2} was then maintained at the measured level throughout the experiments by adjusting exogenous CO_2 in the inspired gas mixture. The EMG and MTA were recorded at paper speeds of 10, 25, and 100 mm/s. After 20 min of steady state at each halothane concentration, the concentration was decreased by 0.5% and maintained for at least 20 min before arterial blood gas analysis and EMG recording at the new concentration, as recommended for the determination of the MAC.[12] Measurement at halothane concentrations less than 1.0% was not possible because most animals woke up.

We measured the peak height of the MTA, respiratory frequency (f), inspiratory time (T_I), and inspiratory duty cycle, or the ratio of T_I and total respiratory cycle duration (T_I/T_{TOT}), using a digitizer and a computer system. At

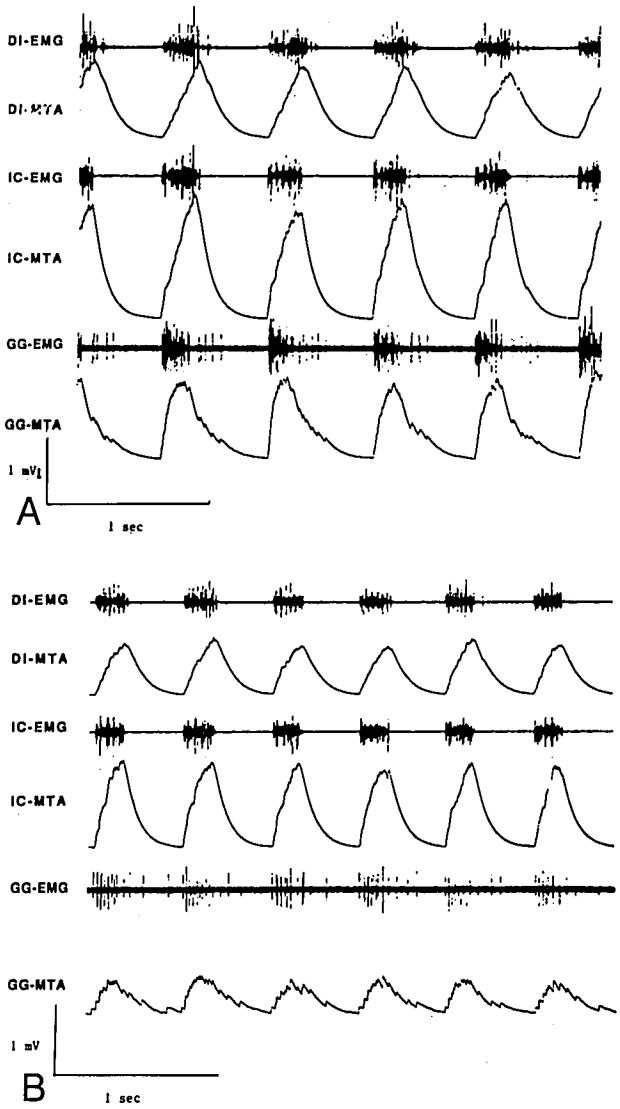

FIG. 2.*A*. Typical recording of EMG and its MTA of three inspiratory muscles at 1.0% halothane (control). DI = diaphragm; IC = external intercostal muscle; GG = genioglossus. *B*. Typical recording of EMG and MTA of the same muscles at 2.0% halothane in the same cat as in figure 2*A*.

least 30 consecutive breaths were measured from the recordings on chart paper at each concentration in each animal, and mean values were obtained. Because peak height is a dimensionless number, it was expressed as percent of control (*i.e.*, the value at 1.0% halothane). Analysis of variance was used to compare the MTA of a single muscle at different concentrations (intraindividual comparison) and to compare percent depression of MTA of different muscles at a given halothane concentration (interindividual comparison). Probability less than 5% was considered statistically significant.

Results

Pa_{CO_2} during spontaneous breathing at 3.0% halothane anesthesia was 61 ± 3.2 mmHg. Thus, it was maintained at approximately 60 mmHg (57–64 mmHg) throughout the study to compare MTA at the same Pa_{CO_2} level. Figure 2 shows a typical recording of the EMG and MTA at 1.0% and 2.0% halothane in one cat.

The peak height from all three muscles decreased in a dose-dependent fashion as the concentration of halothane increased (table 1; fig. 3). Overall, the phasic diaphragmatic activity decreased the least, and genioglossal activity decreased the most; the reduction in intercostal activity was intermediate. The decrease in phasic genioglossal activity was significantly greater than that in diaphragmatic activity ($P < 0.05$) at all concentrations of halothane and significantly greater than the decrease in intercostal activity ($P < 0.05$) at all concentrations except 3.0% (table 1). Average phasic intercostal activity was more depressed than diaphragmatic activity, but the difference was significant only at 3% halothane. In three of seven cats, phasic inspiratory activity of the genioglossus was abolished at halothane concentrations at and above 2.5%.

Respiratory frequency did not change significantly with increasing concentration of halothane. Likewise, neither T_I nor T_I/T_{TOT} changed significantly (table 2).

Discussion

The effect of anesthesia on hypoglossal nerve activity was first described by Hwang *et al.* in 1983.[13] The genioglossus is an external tongue muscle, the contraction of which moves the tongue forward synchronously with the contraction of the diaphragm, thus maintaining upper airway patency during inspiration.[3,7] Recent studies have indicated that the activity of the genioglossus and the hy-

TABLE 1. Effect of Halothane Anesthesia on Inspiratory Muscle Activity* in Cats (n = 7)

Concentration	1.5%	2.0%	2.5%	3.0%
Diaphragm	89.4 ± 3.9	74.6 ± 8.2	65.3 ± 8.3	46.5 ± 7.2
Intercostals	80.1 ± 9.9†	52.6 ± 11.9†	41.0 ± 8.3†	17.6 ± 5.5‡
Genioglossus	45.0 ± 12.9‡	24.8 ± 10.4‡	13.3 ± 9.8‡	6.7 ± 3.9‡

* Expressed as percent of peak height of MTA (mean ± SEM) at 1% (control). All values except for intercostals at 1.5% ($P < 0.1$) are significantly ($P < 0.05$) decreased from 1% controls.

† $P < 0.05$ compared with genioglossus.
‡ $P < 0.05$ compared with diaphragm.

poglossal nerve is attenuated more than diaphragmatic activity by anesthetics, such as halothane, enflurane, and barbiturates,[3,4] and also by alcohol ingestion and sleep.[5,6,14,15]

The current study demonstrated that the phasic activity of various inspiratory muscles is differentially affected in a dose-dependent manner by halothane anesthesia, *i.e.*, the genioglossus is most susceptible, the diaphragm is most resistant, and the response of the intercostals is intermediate. These studies were performed during spontaneous breathing and with minimal interference by instrumentation.

Nishino *et al.*[3,4] showed greater depression of the hypoglossal than the phrenic nerve with halothane and enflurane anesthesia by means of the peak height of the integrated electroneurogram (*i.e.*, MTA), but in paralyzed, mechanically ventilated cats in which vagotomy and tracheostomy had been performed. Hwang *et al.*[13] also demonstrated selective depression of phasic hypoglossal motor activity over phrenic activity by halothane as well as by ketamine and barbiturates, but in decerebrate, paralyzed, and mechanically ventilated cats following vagotomy.

The negative pressure in the upper airway during inspiration[16,17] and the position of the head and neck[10] are important determinants of hypoglossal and genioglossal activity. In studies of animals with an endotracheal tube or tracheostomy, hypoglossal and genioglossal activities are modified because upper airway pressure remains atmospheric. Furthermore, an endotracheal tube, itself as a foreign body, most likely affects genioglossal activity by mechanical stimulation and displacement of the tongue. Vagotomy or carotid denervation also modifies genioglossal activity[17] and respiratory control.[18]

To evaluate the effect on ventilatory control of a given drug, it is essential to maintain chemical stimuli constant. In the current study, Pa_{CO_2} was maintained nearly constant by adjusting CO_2 in the inspired gas. Although sustained moderate hypercapnia is not physiologic, Pa_{CO_2} at approximately 60 mmHg was necessary to consistently recruit genioglossal inspiratory activity.[13] However, because the cats were breathing spontaneously, genioglossal activity was not artificially modified with surgical interventions and instrumentation, and chemical stimuli (CO_2 and *p*H) were constant; *i.e.*, for reasons 1, 2, and 3, our results likely represent near physiologic responses of inspiratory muscles to the increasing depth of anesthesia. It is also likely that under eucapnic conditions, genioglossal activity would be more readily depressed with lower concentrations of halothane than those in the current results.

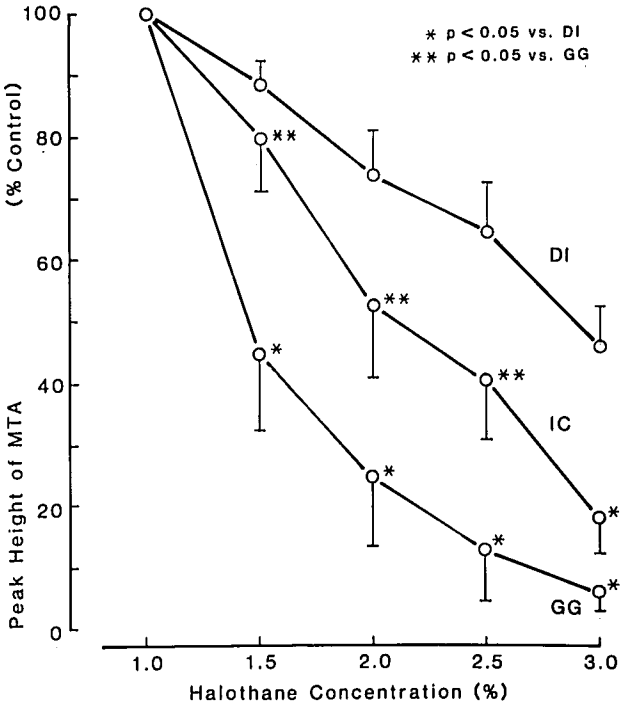

FIG. 3. Decrease in phasic inspiratory muscle activity (peak height of moving time average), expressed as percent change from control (1%), during halothane anesthesia in adult cats. Values are mean ± SEM. *$P < 0.05$ compared with the diaphragm (DI). **$P < 0.05$ compared with the genioglossus (GG).

Our goal in the present experimental setup was to study the effect of halothane on inspiratory muscle activities in spontaneously breathing cats without the influence of any other drugs (barbiturates, muscle relaxants), and with minimal surgical intervention (vagotomy, cortical transsection) or instrumentation (endotracheal tube or tracheostomy, mechanical ventilation). Consequently, the EMG activities at 1% halothane, rather than those in the awake state, were used as control values. A similar approach was used recently by Nishino *et al.*[3] in their study of the phrenic and hypoglossal nerve impulses at different depths of

TABLE 2. Effect of Halothane Anesthesia on Respiratory Frequency (f) and Timing Components (T_I and T_I/T_{TOT}) in Cats (n = 7)

Concentration	1.0%	1.5%	2.0%	2.5%	3.0%
f (/min)	43.2 ± 3.5	41.7 ± 2.3	40.8 ± 1.3	41.8 ± 2.1	39.5 ± 3.5
T_I (s)	0.42 ± 0.08	0.44 ± 0.04	0.46 ± 0.07	0.48 ± 0.07	0.46 ± 0.06
$T_I T_{TOT}$	0.42 ± 0.03	0.44 ± 0.02	0.46 ± 0.02	0.48 ± 0.02	0.46 ± 0.02

Values are expressed as mean ± SEM.

halothane anesthesia in the cat. This approach is justifiable because a later study by the same group of investigators,[4] using alpha-chloralose as basal anesthesia, found a linear dose–response depression of electroneurogram activity between 0% and 2.5% halothane.

The mechanism of differential sensitivity to halothane of the three inspiratory muscles is not clearly understood. Guedel[1] described the selective depression of rib cage movement during diethyl ether anesthesia more than 50 yr ago; yet the effect of anesthesia on the inspiratory muscles has not been quantitated. We found the degree of depression of intercostal muscle activity to be significantly greater than that of the diaphragm at higher concentrations of halothane anesthesia. Although the intercostals and the diaphragm originally receive the same projection from motoneurons in the medulla,[19] they are innervated by different motoneurons in the spinal cord. The phrenic motoneurons in the spinal cord are basically dedicated respiratory neurons with few modifying inputs. However, the intercostal motoneurons receive various respiratory and nonrespiratory inputs from both supraspinal and segmental origins, such as postural and behavioral functions. Thus, it would seem easier to excite phrenic motoneurons than intercostal motoneurons to the threshold. The greater degree of depression in intercostal than in diaphragmatic activity with anesthesia suggests that the site responsible for differential depression may be in the spinal cord rather than in the medulla.

On the basis of previous observations in humans[1,2,6] and in animal experiments,[3,4,5,7,13] it seems likely that similar differences in sensitivity to anesthetics of inspiratory muscles exist in humans. Thus, the possible clinical implications of our study are that patients may be vulnerable to upper airway obstruction in the intraoperative and immediate postoperative periods due to the anesthetic effect on the genioglossus, even when diaphragmatic activity is sufficient to preserve adequate minute ventilation.

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