Dose-dependent Effects of Halothane on the Carbon Dioxide Responses of Expiratory and Inspiratory Bulbospinal Neurons and the Phrenic Nerve Activities in Dogs

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Background: Expiratory bulbospinal and inspiratory bulbospinal neurons in the ventral respiratory group provide drive for thoracoabdominal expiratory and phrenic and thoracic inspiratory motor neurons. Potent inhalational agents such as halothane may have differential effects on inspiratory and expiratory neurons, but detailed studies comparing neurons at a homologous level are lacking.

Methods: The dose-dependent effects of anesthesia with 1.0–2.5 minimum alveolar concentration halothane on the CO₂ responses of single expiratory and inspiratory bulbospinal neurons of the ventral respiratory group and on phrenic neural activities were studied in nonanesthetized, anesthetized, paralyzed, vagotomized dogs. Hyperventilation with O₂ and the addition of CO₂–O₂ mixtures were used to produce low, medium, and high steady-state levels of central CO₂ drive.

Results: Peak neuron discharge frequency decreased progressively with increasing halothane dose at all levels of CO₂ drive for both types of neurons. The sensitivities of inspiratory and expiratory bulbospinal neuronal activities to halothane were not significantly different from one another, whereas the sensitivity to halothane of the peak phrenic activity was markedly greater than those of the neurons. Increasing halothane dose caused a downward, predominantly parallel shift of the CO₂ response curves. Phrenic nerve activity also showed a decrease in slope of the CO₂ response.

Conclusions: The activities of respiratory premotor neurons are less depressed by increasing doses of halothane than is phrenic nerve activity. The greater depression of phrenic activity may result from additional anesthetic actions on the efferent motor pathways, resulting in decreased descending synaptic inputs to phrenic motor neurons. (Key words: Anesthetics, volatile; halothane. Brain: expiratory bulbospinal neurons; inspiratory bulbospinal neurons; ventral respiratory group. Nerves: phrenic; vagus. Ventilation: central carbon dioxide ventilatory response.)

RECENTLY we showed that inspiratory neural activity measured as phrenic neural output is more depressed than extracellularly recorded expiratory premotor neuron activity by increasing doses of isoflurane.¹ We suggested that the greater depression of phrenic neural output resulted from anesthetic actions on central inspiratory neurons and their efferent motor pathways. In particular the synapses between premotor and phrenic motor or spinal interneurons may be prime targets for additional anesthetic depression.² We further hypothesized inspiratory premotor neurons may be more sensitive to the depressant effects of potent inhalational anesthetics than expiratory premotor neurons.¹ Such a differential depressant effect by halothane on central respiratory neurons in the rostral ventral respiratory group (VRG) has been reported.³ Most of those neurons could not be antidromically activated from the cervical spinal cord and were thought to be laryngeal (vagal) motor neurons concerned with upper airway control rather than bulbospinal motor neurons.

In the current study we directly compared the responses of inspiratory bulbospinal (IBS) and expiratory bulbospinal (EBS) premotor neurons of the VRG with regard to dose-dependent depression by halothane. The neurons all were located caudal to the obex in the region of the nucleus retroambiguus. We also examined the dose-dependent effects of halothane on the CO₂ responses of these neurons and phrenic neural activities in nonanesthetized, anesthetized, paralyzed, vagot-
omized dogs whose lungs were mechanically ventilated. The purposes of the study were (1) to establish a dose–response curve for the effect of halothane on the electrical activity of IBS neurons, EBS neurons and the phrenic nerve; (2) to examine how increasing doses of halothane alter the CO₂ responses, mediated by central chemosensors, of these neurons; and (3) to test the hypothesis that increasing anesthetic depth has a differential effect on phrenic neural, inspiratory neuronal and expiratory neuronal activities.

Materials and Methods

Surgical Preparation

This research was approved by the Medical College of Wisconsin Animal Care Committee and conformed with standards set forth by the National Institutes of Health. The surgical preparation and experimental setup were described in detail in our previous publication. In the current studies we used halothane rather than isoflurane and also sampled inspiratory neurons. Thirty-eight adult mongrel dogs (weight 8–16 kg) were studied under halothane monoanesthesia. Airway CO₂ and halothane concentrations were continuously recorded with an infrared analyzer (POET II, Criticare Systems, Milwaukee, WI) calibrated before each experiment. The lungs were mechanically ventilated with O₂, end-tidal halothane concentrations of 1.3–1.8 minimum alveolar concentration (MAC) were used for surgery. The femoral vessels were cannulated for blood sampling, blood pressure recording, administration of maintenance fluids (isotonic saline with 0.1 mEq·ml⁻¹ NaHCO₃ at 6–8 ml·kg⁻¹·h⁻¹). Phenylephrine (0.5–5.0 μg·kg⁻¹·min⁻¹) was infused when necessary to keep mean arterial pressure above 75 mmHg. Additional NaHCO₃ to correct metabolic acidosis was given as needed. Esophageal temperature was maintained at 38 ± 0.5°C with a servo mechanism-controlled heating pad. The dogs were positioned prone in a stereotaxic apparatus (1550, David Kopf Instruments, Tujunga, CA) with the head flexed ventrally by 30°. Bilateral, dorsolateral neck dissections were performed. The dorsal medulla oblongata was exposed by occipital craniotomy. The right central C5 phrenic nerve rootlet was cut distally, desheathed, immersed in a mineral oil pool and placed on bipolar platinum electrodes. The animal was paralyzed with a 0.1 mg·kg⁻¹ bolus and continuous infusion of vecuronium (0.15 mg·kg⁻¹·h⁻¹). Inputs from pulmonary stretch receptors and aortic arch chemoreceptors were removed by bilateral vagotomies. A bilateral pneumothorax was performed to minimize brainstem movement and to eliminate phasic inputs from chest wall mechanoreceptors.

In addition, in three animals a C3 laminectomy was performed and the dura mater was opened widely to expose the dorsal surface of the spinal cord to allow for insertion of microelectrodes for antidromic activation of neurons. Four tungsten microelectrodes, mounted as two pairs on a holder, one pair on each side of the spinal cord, were inserted to a depth of 4–5 mm. The interelectrode distance for each pair was 1.75 mm. The distance between pairs, as measured between the inner electrodes of each pair, was 2.75 mm. All four electrodes were connected in parallel. A constant-current stimulator was used to deliver cathodal current pulses, and a single, remotely located anodal electrode was used.

Recording Techniques and Data Acquisition

The following variables were continuously recorded on a polygraph (7, Grass, Quincy, MA). Efferent phrenic nerve activity from the C5 rootlet was amplified with a band pass of 0.1–3 kHz, full-wave rectified, and low-pass-filtered with a time constant of 100 ms to obtain a moving time average of the activity. Bulbospinal neuronal activity was recorded extracellularly with a tungsten microelectrode (tip diameter 1 μm, impedance 10 MΩ at 1,000 Hz) inserted through the dorsal medulla 2–5 mm caudal to the obex, 2.5–4.5 mm lateral to the midline, to a depth of 2–4.5 mm. The amplified output of the microelectrode was monitored on a cathode ray oscilloscope, and an amplitude-time window discriminator was used to generate a standard pulse for each neuronal spike. Pulses were counted during 100-ms intervals, and the spike frequency was displayed on the polygraph through a digital-to-analog converter. IBS neurons were found mainly in the rostral part of our probing region (1.5–2.5 mm caudal to the obex) while EBS neurons were distributed throughout the caudal VRG. Also measured were arterial blood pressure, airway CO₂ concentration, airway halothane concentration, and tracheal pressure. Representative samples of all variables were also recorded on an eight-channel digital video recorder (3000A, Vetter, Rebersburg, PA) for later computer assisted analysis.


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Protocol
After completion of surgery the end-tidal halothane concentration was adjusted to 0.9% (1 MAC) and maintained for at least 1 h before recordings. The steady-state response of the neurons to three target levels of arterial CO₂ tension (PaCO₂) (low 25–35 mmHg, medium 40–50 mmHg, and high 60–70 mmHg) was obtained at four levels of halothane anesthesia (0.9% = 1 MAC; 1.35% = 1.5 MAC; 1.8% = 2 MAC; and 2.25% = 2.5 MAC) during hyperoxia (arterial O₂ tension > 400 mmHg) for as many neurons as possible.

After the localization of a stable respiratory neuron, the lungs were hyperventilated at a fixed rate of 20 breaths/min to reach the low PaCO₂ target range. CO₂ was admixed to produce the medium and high PaCO₂ levels. Stable end-tidal CO₂ concentrations were maintained for a minimum of 5 min before data collection. Arterial blood gases were measured to confirm that the PaCO₂ target range had been reached. End-tidal halothane concentrations were kept constant for at least 15 min. A complete neuronal study protocol required 5–6 h. Halothane doses were always administered in increasing concentrations. At the completion of the protocol the anesthetic depth was decreased to the original baseline level (1 MAC) to obtain end control values at the high CO₂ level after minimum anesthetic washout times of 30 min. In general, one or two neurons per animal were studied. No specific attempt was made to obtain an inspiratory and an expiratory neuron in the same animal. All animals were killed with 4% halothane and a subsequent KCl bolus after data collection.

Data Analysis
Any neuron for which the high PaCO₂ data point at 1 MAC halothane anesthesia (baseline) and at least one additional data point (at a different PaCO₂ or at the next anesthesia level) could be obtained was used for data analysis. A complete anesthetic dose response was obtained for 10 of 28 EBS and 12 of 32 IBS neurons. In these cases, end control values at the baseline anesthetic level at high CO₂ were compared with the initial baseline values (Student's t test).

Data for all respiratory parameters were averaged over five to ten respiratory cycles at each data point. The averaged data for peak phrenic neural activity (PPA) and peak respiratory neuron discharge frequency (Fr) were normalized to their respective values at 1 MAC halothane anesthesia at high PaCO₂ for each respiratory neuron and phrenic nerve response. The normalized data and the absolute data for respiratory rate, inspiratory duration (Ti), and expiratory duration (Te) were analyzed by applying a two-way analysis of variance (ANOVA) technique (SuperAnova, Abacus Concepts, Berkeley, CA) that allowed for unbalanced cell numbers, where the factors were anesthetic dose and PaCO₂ level. Scheffé's S procedure was used for post hoc comparisons for single factors, and when significant interactions between factors were detected, the Bonferroni procedure was used for multiple comparisons between levels of one factor at various levels of the second factor. At each PaCO₂ drive level, the effects of increasing halothane dose level were tested through trend analysis that used orthogonal linear contrasts within the context of a one-way analysis of variance. Data are presented as mean values with standard errors unless otherwise stated. Probability levels of P < 0.05 were used to indicate statistical significance.

Results
Data from 28 expiratory neurons and 32 inspiratory neurons in 35 dogs were recorded and analyzed. Antidromic mapping was performed in an additional three animals. This technique showed that 15 (88.2%) of 17 inspiratory neurons and 21 (80.8%) of 26 expiratory neurons in the medullary region we sampled could be antidromically activated, indicating that they had axons descending to the spinal cord. Thus they were bulbospinal premotor neurons.

Figure 1 shows the effect of varying CO₂ drive at a steady-state halothane level on phrenic nerve (upper

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two traces) and on inspiratory neuron activity (lower two traces). The phrenic nerve activity is shown as "raw" activity (after amplification and filtering) as seen on the oscilloscope and after full-wave rectification as a moving-time average, or phrenic neurogram (PNG), as recorded on the polygraph. Inspiratory neuronal activity is shown as a train of uniform raw spikes (as seen on the oscilloscope) and after amplitude-time window discrimination and pulse counting per 100 ms, as discharge frequency (as recorded on the polygraph). Decreasing CO₂ drive leads to a decrease in PPA height as well as inspiratory peak Fₙ.

Figure 2 illustrates the effect of increasing halothane dose on phrenic nerve activity and another inspiratory neuron at the medium CO₂ drive level. With increasing anesthetic dose, PPA declines markedly. In contrast, the peak inspiratory Fₙ shows a less marked decrease. Additionally, figure 2 demonstrates Tₐ decreases with increasing depth of anesthesia.

Effect of Halothane Dose on the Electrical Activity of the Respiratory Neurons and the Phrenic Nerve

Figure 3 summarizes the effect of increasing halothane dose on the normalized peak Fₙ of all inspiratory and expiratory neurons as well as the PPA for the three CO₂ drive levels. The number in the bars indicate the actual number of neurons studied at each data point. The phrenic data during expiratory and inspiratory neuronal recordings were pooled. The mean (±SE) peak Fₙ values for 32 inspiratory and 28 expiratory

neurons at 1 MAC and high CO₂ drive were 123.6 ± 8.40 Hz and 122.6 ± 6.52 Hz, respectively. Actual measured mean PₐCO₂ levels for expiratory and inspiratory neurons respectively were (mean ± SD): high: PₐCO₂ = 67.0 ± 3.5 (expiratory), PₐCO₂ = 67.0 ± 3.1 (inspiratory); medium: PₐCO₂ = 46.5 ± 2.4 (expiratory), PₐCO₂ = 47.0 ± 3.9 (inspiratory); low: PₐCO₂ = 28.4 ± 3.2 (expiratory), PₐCO₂ = 30.8 ± 4.9 (inspi-
Do Increasing Doses of Halothane Cause Differential Depression of Inspiratory and Expiratory Bulbospinal Ventral Respiratory Group Neurons?

Because our experimental model did not allow us to study the animals in an anesthetic-free state, we could not examine whether the presence of halothane per se causes differential depression of the two neuron types. To delineate any possible differential effects of increasing doses of halothane anesthesia on the two groups of VRG neurons, we calculated the amount of depression for each neuron and the corresponding phrenic nerve activities between the 1 MAC and 2 MAC halothane levels at each CO$_2$ drive level and used it as a measure of sensitivity to the anesthetic: sensitivity = (activity at 1 MAC − activity at 2 MAC)/(2 MAC − 1 MAC). Figure 4 summarizes these data. This variable was analyzed by a two-way ANOVA with main factors of neural activity type and CO$_2$ drive level. PPA is significantly more depressed (greater sensitivity) by the 1 MAC dose increase in halothane depth than is EBS or IBS neuronal activity at the high and medium CO$_2$ drive level. However, a statistically significant difference in the sensitivity to halothane between EBS and IBS neurons could not be found.

Effect of Central CO$_2$ Drive on the Activity of the Respiratory Neurons and the Phrenic Nerve

The CO$_2$ response curves for peak neural activities for the four doses of halothane were analyzed. There was a PaCO$_2$-dependent increase in the peak activities of EBS neurons, IBS neurons, and the phrenic nerve at all halothane levels (P$_{TREND}$ < 0.0001 all doses except 2.5 MAC, P < 0.05, for all three variables, as shown in fig. 5). For each neuron and the corresponding phrenic nerve, the CO$_2$ response sensitivities (sensitivity = Δ peak activity/Δ PaCO$_2$, in percentage per millimeter mercury) for the low (low to medium PaCO$_2$) and high (medium to high PaCO$_2$) PaCO$_2$ ranges were calculated and analyzed by a two-way ANOVA with halothane dose and PaCO$_2$ range as factors. At 1 MAC, the CO$_2$ sensitivities for the EBS, IBS, and phrenic neural activities for the low CO$_2$ range were 1.37 ± 0.204, 2.47 ± 0.314, and 2.34 ± 0.294%/mmHg, respectively. For the high CO$_2$ range, the corresponding sensitivities were 0.748 ± 0.148, 1.21 ± 0.127, and 1.27 ± 0.156%/mmHg, respectively.

For all three types of activities, there was a dose-dependent, downward shift in the CO$_2$ response curves, and at 1 MAC (only) the low range CO$_2$ sensitivities were significantly greater than those of the high range. For the EBS neurons, there were no changes in the CO$_2$ sensitivities for either range, while, for the IBS neurons, there was a marked dose-dependent reduction of the low CO$_2$ range sensitivities (P$_{TREND}$ < 0.0005), but no change in the high range sensitivities. For the pooled PPA responses, there were dose-dependent reductions in the sensitivities for both the low and high CO$_2$ ranges (P$_{TREND}$ < 0.0001 for both).
HALOTHANE DEPRESSION OF RESPIRATORY PREMOTOR NEURONS

The differential effects of halothane on EBS and IBS neuronal and phrenic activities are also illustrated in figure 5 for the 1 MAC and 2 MAC halothane doses. The graph underscores the marked anesthetic induced depression of phrenic nerve activity causing a large downward shift and a reduction in the sensitivity of the CO₂ response curve, while EBS and IBS neuronal activities show relatively more resistance to halothane.

In summary, our analyses suggest that the main effect of halothane dose is a parallel shift in the CO₂ response curves of neuronal and phrenic activities. In addition, CO₂ sensitivity is reduced by increasing halothane dose for the low CO₂ range for IBS neuron as well as phrenic nerve activity. Phrenic nerve activity also shows a dose-dependent reduction in CO₂ sensitivity for the high CO₂ range.

Effect of Halothane on Inspiratory Drive
It has been shown that both the initial rate of rise, as well as, the apneustic plateau level of the PNG depend on the prevailing arterial O₂ tension and PₐCO₂. During cupneta there is no plateau phase, and the PNG is ramp-like. The PPA near the end of the inspiratory phase can then be used as an index of inspiratory drive as long as T₁ remains relatively constant. The PPA parameter is easy to quantify. However, because changes in halothane concentration alter T₁, we used two additional measures of inspiratory drive in our analyses: the PNG slope at 100 ms after the onset of phrenic activity (SLP (0.1)), and the average PNG slope, PPA/T₁.

For 33 neuronal studies, each of these three parameters was normalized to the high drive, 1 MAC condition, and then analyzed using a two-way ANOVA with main factors of halothane level and CO₂ drive level. Both of these main effects were significant for all three parameters (0.0001 < P < 0.001). The data of figure

Fig. 5. CO₂ response curves of peak expiratory bulbospinal neuron (EBSN) discharge frequency (Fₑ), peak inspiratory bulbospinal neuron (IBSN) Fₑ, and peak phrenic nerve activity (PHR) for the 1 MAC and 2 MAC halothane doses. Each point = mean ± SE. The main effect of increasing halothane dose was a parallel, downward shift in the CO₂ response curves for all three activities. The anesthetic induced marked depression of phrenic nerve activity, whereas the EBSN was relatively resistant.

Fig. 6. Comparison of three indices of inspiratory drive derived from the phrenic neurogram at the three CO₂ drive levels: peak phrenic nerve activity (PPA) (solid bars), slope of the phrenic neurogram 100 ms after the onset of phrenic activity (SLP (0.1)) (open bars), and average slope of phrenic nerve activity (PPA/inspiratory duration [PPA/T₁]) (stippled bars). All three indices of inspiratory drive showed a dose-dependent depression and were found to be closely correlated.

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6 clearly demonstrate a graded dose-dependent and inverse drive-dependent reduction in these indices, with the effects being most prominent for PPA and least prominent for PPA/Ti. For each study, the mean (±SE) correlation coefficients (r values) obtained from plots of PPA versus SLP (0.1), PPA versus PPA/Ti, and PPA/Ti versus SLP (0.1) were 0.885 ± 0.034, 0.845 ± 0.033, and 0.955 ± 0.0086, respectively.

**Effect of Halothane Dose and Central CO₂ Drive on Respiratory Timing**

Data from all neuronal studies were pooled to calculate the average respiratory rate of the vagotomized animals (fig. 7, upper). The average respiratory rates (mean ± S.E.) at the 1 MAC halothane level were 21.4 ± 2.5, 24.6 ± 1.7, and 21.3 ± 1.2 breaths/min for the low, medium, and high CO₂ drive levels, respectively. Increasing halothane dose caused a gradual reduction in respiratory rate at all CO₂ drive levels. A two-way ANOVA indicated significant main effects for both factors, halothane dose and CO₂ drive level (P < 0.0001 for both), as well as a significant interaction between these factors (P = 0.0012). Trend analysis of the halothane dose–response data at each drive level indicated significant linear trends for all three CO₂ drive levels (Pₚₚₑₑₑₑₑₑₑₑₑₑₑₑ < 0.0001). An increase in central CO₂ drive caused a moderate increase in respiratory rate, which was most pronounced between the low and medium CO₂ drive levels at all halothane doses (P < 0.0001). The interaction between factors CO₂ drive and halothane dose (P < 0.0012) appears to be due to the presence of data at the 2.5 MAC level that converge to zero breaths per min.

For the same subset of studies for which comparative inspiratory drive data were obtained, Tₑ and Tₑ were calculated by computer off-line. Tₑ decreased significantly with increasing halothane dose (P < 0.0001), and with changes in CO₂ drive level (P < 0.0001) (fig. 7, middle). Tₑ increased significantly with increasing halothane dose (P < 0.0001). At the high drive level, there was a significant linear trend for Tₑ versus MAC level (Pₚₑₑₑₑₑₑₑₑₑₑₑₑ < 0.0001). At all MAC levels, there were no differences between Tₑ values for the medium and high drive levels. An increase in Tₑ with decreasing CO₂ drive is due to some animals taking very few breaths at the 2.0 MAC, low CO₂ drive level before becoming apneic at the 2.5 MAC level. That pattern led to long phases of expiratory apnea, as supported by tonic expiratory neuron discharge.

![Graphs showing effect of halothane dose and central CO₂ drive on respiratory timing.](image)

**End Controls**

End control values at the baseline anesthetic level and high CO₂ drive were obtained for 10 of 28 expiratory and 12 of 32 inspiratory neurons and their corresponding phrenic nerve responses. Peak expiratory Fₚₑₑₑₑₑₑₑₑₑₑₑₑ (93.7 ± 7.4%) and peak inspiratory Fₑₑₑₑₑₑₑₑₑₑₑₑ (93.5 ± 4.3%) were not significantly different than the initial baseline values. End control PPA (86.6 ± 4.6%) recovered incompletely and was lower than the initial baseline value (P = 0.0037).

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HALOTHANE DEPRESSION OF RESPIRATORY PREMOTOR NEURONS

Discussion

Our results show that halothane anesthesia causes a dose-dependent depression of the activity of IBS and EBS neurons. Increases in central CO₂ drive can only partially offset the depressant effects of increasing anesthetic depth. The activities of EBS and IBS neurons in the caudal VRG appear to be depressed by halothane to the same degree, as we were not able to confirm a statistically significant differential effect of a 1 MAC dose increase of halothane on the two groups of VRG premotor neurons. Phrenic nerve activity was considerably more depressed than inspiratory neuron activity with increasing halothane concentration. Presumably the anesthetic effects on the phrenic neural output result from anesthetic actions on the central inspiratory neurons, as well as, on their motor efferent pathways including the motor premotor neurons themselves. The motor efferent pathways between IBS neurons and the phrenic motor neurons are connected by at least one additional synapse, and synaptic transmission has been shown to be particularly vulnerable to depression by potent inhalational anesthetics. Thus, less excitatory inputs from the premotor neurons reach the phrenic motor neurons and they might then not be depolarized enough to reach firing threshold.

An alternative explanation of this phenomenon suggests that the relatively greater depression of phrenic nerve activity might well be the result of the non linear physiological process of derecruitment common to motor neurons. However, evidence that may support an additional depressant effect of halothane at the level of the phrenic motor neurons or their presynaptic inputs, is the comparison of the relations between PPA and peak IBS neuronal activity when neuronal excitability was altered by changes in P̄CO₂ versus changes in halothane concentration. Based on the mean data of figure 3, this relation as a function of CO₂ drive at the 1 MAC level is linear, with a slope near unity (1.16%/%). However, this same relation, as a function of halothane dose at the high CO₂ drive level, is much steeper (2.28%/%). Both relations have a common data point (100%, 100%) for the 1 MAC, high CO₂ drive condition.

The IBS neurons, themselves, appear to be fully recruited over the entire CO₂ drive range, which extended down to an average P̄CO₂ of 31 mmHg, and even at this low drive level, a halothane level of at least 2 MAC was required before some of these neurons became silent. Thus it appears that these neurons normally contribute to ventilatory drive through increases in their discharge frequency. Because our search for these IBS neurons took place at 1 MAC under normo- to hypercapnic conditions, our sampling did not necessarily favor neurons with low recruitment thresholds. However, it is possible that many vulnerable IBS neurons may have been eliminated in going from the awake state to 1 MAC halothane. Our experiments do not allow us to assess this issue. However, in a decerebrate cat model, Grelot and Bianchi found that the peak activities of dorsal respiratory group (DRG) and VRG inspiratory neurons were depressed to the same extent as that of the phrenic nerve, where 1–2% halothane reduced the PPA to approximately 86% of the “awake” control. Thus it is likely that VRG IBS neurons in dogs, many of which are phrenic premotor neurons, would not have been eliminated in our study by 1 MAC halothane anesthesia.

Afferent-mediated Inputs to Bulbospinal Respiratory Neurons

To selectively study the contribution of the central chemoreceptors in the form of CO₂ response curves, pulmonary stretch receptor afferent inputs were eliminated by vagotomy and the carotid chemoreceptors were functionally denervated by hyperoxia. Respiratory activities were only recorded at steady-state levels of CO₂ drive after a minimum equilibration of 5 min, which is equivalent to three times the response time constant of the central chemoreceptors to CO₂.

The observed dose-dependent, downward shift and reduction in sensitivity (slope) of the CO₂ response relations suggest that both central inspiratory and expiratory drives are depressed by halothane. Downward, parallel shifts are associated with changes in threshold levels of activity, which are additive in nature. Such shifts may result from decreases in membrane excitability at the level of the bulbospinal neuron or upstream in intermural neurons that relay drive from the central chemoreceptors. In addition, a dose-dependent reduction in the slope of the neuron activity—P̄CO₂ relations for the low-drive range occurred. Slope changes are functionally associated with multiplicative gain changes, which may be due to a reduction in the effectiveness of synaptic transmission and synaptic modulation, and the central CO₂ transduction process.

Critique of the Methods

The current studies were carried out under steady-state conditions for halothane concentrations, P̄CO₂,
and hemodynamics. Due to the relatively long washout times of the agent, our protocol used a progressive increase in halothane concentration rather than a randomization of the different concentrations. However, end control values obtained for a third of the neurons (10 of 28 expiratory and 12 of 32 inspiratory neurons) were not statistically different from the initial baseline values. This suggests that central neuronal activity had fully recovered from the effects of deep halothane anesthesia after a 30-min washout period, and that our animal preparation was stable. A lack of complete recovery of the peak PNG (86.6% of baseline) is most likely due to changes in the electrode-nerve interface common to recordings from multifiber preparations.

Our monoanesthetic approach avoids the confounding effects of background anesthetics, but this in vivo model does not allow us to study the animals in a drug-free state. This shortcoming does not allow us to make categorical assessments of the halothane effect per se on the two types of neurons studied. If the baseline, 1.0 MAC, halothane dose was able to cause a major differential depression of one neuron group versus the other, we would not be able to reliably assess this. In our previous study, the dose–response curves for PPA and peak expiratory Fv were monotonic, decreasing functions of iso-urethane concentration from 0.5 to 2.5 MAC, with no suggestion of altered differential sensitivities in the 0.5–1.0 MAC range. Tabatabai et al. showed that the activity of DRG inspiratory neurons was depressed to about 20% of “no-anesthetic” control after a 20-min exposure of 1% halothane (1.15 MAC) in decerebrate, vagotomized, paralyzed, mechanically ventilated cats. These authors suggested that the nucleus tractus solitarius may serve as a site of action of the halothane-induced respiratory depression. However, this degree of depression (i.e., 20% of control) of DRG inspiratory neurons may not be representative of the phrenic activity: Hwang et al., using a similar decerebrate cat model, found very little depression of the PPA with 0.5% halothane, whereas hypoglossal motor activities were markedly suppressed, and Gautier et al. found tidal volume decreased by 33% with 1% halothane in decerebrate, spontaneously breathing cats. Unfortunately, no comparable data are available for expiratory premotor neurons in a decerebrate model, so that we do not know whether halothane per se depresses expiratory premotor neuron more or less than inspiratory neurons. Dose increases beyond the 1.0 MAC level, however, do not show a statistically significant difference in depression between the two groups of neurons (fig. 4).

Although it would be highly desirable to carry out these studies with an anesthetic-free control state, the extensive surgical preparation required to measure neural activities does not permit the study in awake animals. Even under light anesthesia (e.g., 0.5 MAC), it is extremely difficult to maintain stable neuronal recordings during alterations in CO2 drive. Therefore, we decided to select the 1.0 MAC dose as the baseline for our protocol. We have previously discussed the two main alternatives to our monoanesthetic approach: decerebration or parenteral background anesthesia. Preliminary anatomical studies showed us that, unlike the cat, decerebration in the dog while maintaining a stable preparation is not easily feasible. Furthermore, decerebration does not guarantee either that the results will be equivalent to the awake state, as all descending central inputs rostral to the brain stem are eliminated. Some of these central inputs may considerably influence the respiratory centers in the awake state, and it is conceivable that they might be partially preserved under light halothane anesthesia while being completely eliminated by decerebration.

We have shown that a commonly used thiopental based, background anesthetic leads to severe respiratory depression when combined with relatively low doses of potent inhalational anesthetics and thus is not a useful alternative. However, we routinely use a thiopental based anesthetic infusion when we perform physiological reflex studies of respiratory neurons. These animals tend to be relatively hypertensive even though they appear well anesthetized as judged by lack of movement and lid reflexes while not paralyzed. Fv,s of the same group of respiratory neurons and phrenic nerve activities are similar in these thiopental anesthetized animals as in animals under 1 MAC halothane monoanesthesia and respond similarly to changes in central CO2 drive.

Significance of the Phrenic Neurogram
The PNG represents the overall neural output of the phrenic motor neuron pool, which in turn receives inputs from the IBN neurons of the DRG in the ventral-lateral nucleus tractus solitarius and from inspiratory neurons in the VRG. It is generally accepted that

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the PNG is a good indicator of the overall central inspiratory neural output from the brain stem for the inspiratory muscles of the chest wall and the diaphragm. Peak height of the PNG correlates well with the magnitude of the tidal volume and is accepted as a representative index of tidal volume, provided that no changes in end-expiratory volume occur. However, some controversy exists at least in humans as to whether potent inhalational agents differentially depress the rib cage contribution to ventilation more than diaphragmatic activity. At 1 MAC halothane only the rib cage contribution to ventilation was markedly depressed. No similar data are available for the dog, but by focusing on the PNG, we restricted our assessment to the central inspiratory drive ultimately conveyed to the diaphragmatic component of ventilation, which would presumably be least depressed at our 1.0 MAC halothane baseline level. We would like to stress that this study is not concerned with the peripheral effects of halothane at the neuromuscular junction and spinal cord integration, but with its central effects on neural activities.

Bulbospinal Neurons of the Ventral Respiratory Group

In previous studies, we have shown that 29 of 31 inspiratory neurons in the region of the nucleus retroambigualis showed positive collision tests when antidromically activated. A positive collision test results if an evoked spike from a stimulating electrode, placed in the spinal cord near the axon of a premotor neuron, travels antidromically toward the soma of the premotor neuron, and somewhere along the axon encounters a spontaneous spike traveling in the opposite direction (orthodromically) so that both will be canceled. Therefore, no response to the stimulus will be recorded. If on the other hand, the spontaneous and the evoked spikes do not travel along the same axon, they will not interfere with each other resulting in no collision. Thus, a positive collision test for our respiratory neurons allows us to confirm that a particular neuron has an axon descending in the spinal cord.

In the current study, antidromic activation tests were performed in three animals and positive collision tests were obtained in 21 of 26 expiratory and 15 of 17 inspiratory neurons, indicating that approximately 90% of the expiratory and inspiratory neurons in our sample were bulbospinal. Because we were interested in examining the effect of increasing doses of halothane on homologous neuronal groups, the bulbospinal inspiratory and expiratory neurons of the VRG were chosen. Both types of neurons in this region are thought not to be part of the respiratory pattern generating neurons, but rather follower neurons that provide drive to the antagonistic respiratory muscles of the chest cage, abdomen and diaphragm via spinal motor or interneurons. Any significant differential effect of increasing halothane dose on these two neuron types could suggest that a major contributor of anesthetic depression is that due to differential vulnerability rather than mainly or exclusively a consequence of depression at multiple synaptic sites in the efferent motor pathways.

VRG EBS neurons mainly project to the contralateral spinal cord and have been demonstrated to excite thoracic and abdominal expiratory motoneurons monosynaptically. They also provide inhibition, presumably via interneurons, of thoracic inspiratory motoneurons during the inspiratory phase of breathing. As previously discussed, EBS neuronal activity is controlled by multiple inputs.

The VRG IBS neurons from approximately 4 mm rostral to 1 mm caudal to the obex, corresponding anatomically with the nucleus ambiguous and retroambigualis, have been shown to project predominantly to the contralateral phrenic motoneurons (C4–C6) and to the contralateral thoracic ventral horn regions that contain intercostal inspiratory motoneurons (T1–T12). Thus, many VRG IBS neurons are premotor neurons that are important in controlling respiratory movements of the diaphragm and inspiratory intercostal muscles. Cross-correlation analysis of VRG inspiratory neuronal impulse activity with whole phrenic nerve activity showed that approximately 40% of these neurons make mono- or polysynaptic connections with the phrenic motor neurons. These IBS neurons also receive inputs from multiple peripheral, as well as, central respiratory afferent fibers. All the IBS neurons that we studied were located in an area 1.5–2.5 mm caudal from the obex and could be either considered a part of the nucleus retroambigualis, or alternatively, of the intermediate region of the VRG. The most caudal portion of the VRG is reported to contain predominantly expiratory neurons. In contrast, of the neurons recorded in the DRG, approximately 96% have inspiratory modulated discharge patterns, and approximately 4% have expiratory patterns. About 50–90% of the DRG inspiratory neurons project down the spinal cord of the cat. In our effort to compare only homologous groups of neurons we elected not to study these neurons, and our results cannot be automatically
applied or extended to any other respiratory neuron groups.

Previous Studies

We have previously discussed in detail those studies that examined the effects of potent inhalational agents on respiratory neurons.\(^1\) It needs to be emphasized that use of background anesthesia,\(^2\) lack of steady-state anesthetic levels,\(^3\) and heterogeneity of respiratory neurons and nerve responses examined\(^4\) make comparisons difficult.

Our findings for VRG IBS neurons differ from those of Grelet and Bianchi, who showed that inspiratory neurons of the retrofacial nucleus were more depressed than PPA by halothane.\(^5\) The majority (36 of 40) of the inspiratory neurons in their study could not be antidromically activated from the spinal cord, suggesting that they were not bulbospinal.\(^3\) They speculated that some of the inspiratory retrofacial nucleus neurons were part of the system that drives the upper airway respiratory motor neurons, responsible for abduction of the vocal cords during inspiration. This system has been shown to be suppressed more by anesthesia than the bulbospinal-phrenic system.\(^6\) Grelet and Bianchi also reported that 31 inspiratory and 11 expiratory neurons in both the DRG and VRG, outside the retrofacial nucleus, showed decreases in mean firing frequency to anesthesia that paralleled the declines in PPA.\(^5\) However, they did not specify if any of these neurons were bulbospinal or caudal VRG neurons. This group of neurons appears more depressed than the neurons we studied: the activity of the former declined at the same rate as the phrenic nerve activity, whereas the VRG IBS neurons in our dogs were less depressed than the phrenic activity. It is possible that their use of an intramuscular background anesthesia before midcollicular decerebration may have had residual confounding effects.

Halothane versus Isoflurane

Comparisons of the effects at multiples of MAC between potent inhalational agents should be regarded with caution because MAC multiples of one agent do not necessarily represent comparable depths of anesthesia with another agent.\(^4\) By definition, however, anesthetic depth is equal between inhalation agents at the 1 MAC dose level. Although we cannot be certain that 1.0 MAC halothane causes an equal depression of our respiratory neural parameters as 1.0 MAC isoflurane because of the lack of an agent free baseline state as discussed earlier, a comparison of the absolute peak \(F_{\text{es}}\) of the EBS neurons at the 1.0 MAC dose, high CO\(_2\) drive level showed no statistically significant difference between the two agents. The peak \(F_{\text{es}}\) under above conditions for 28 EBS neurons with halothane was 122.6 ± 34.5 Hz (mean ± SD) and for 30 EBS neurons with isoflurane 118 ± 40.3 Hz (\(P = 0.7052\)). Because all values in both our isoflurane and the current study have been normalized to the 1.0 MAC dose, high CO\(_2\) drive level, a comparison between both agents at least for the EBS neurons is readily accomplished, and ANOVA indicates that overall there is no significant difference between the agents as far as these neurons are concerned (\(P = 0.3133\)). Because PPA is expressed in arbitrary units, a comparison between agents is not possible. However, if one assumes that PPA is depressed roughly to the same extent at the high CO\(_2\) drive and 1.0 MAC dose by the two agents, then increasing doses of isoflurane depressed phrenic nerve activity significantly more (\(P = 0.0212\)) than halothane, but the magnitudes of these differences were rather small. Nonetheless, our studies suggest that isoflurane depresses phrenic nerve activity slightly more than halothane at all CO\(_2\) drive and anesthetic levels examined. These results are consistent with data gathered in humans, which showed that isoflurane produced larger dose-dependent elevations in \(P_{\text{aco}}\) than halothane at the same MAC multiples.\(^4\) However, the human studies measured overall ventilation, so that anesthetic effects on the central controller and the peripheral components of the respiratory system cannot be separately assessed.

Inspiratory Drive Effects

In the current studies, as well as in our previous studies with isoflurane we used neuronal and neural activities recorded under open-loop conditions. By controlling pertinent variables and removing confounding afferent inputs to the respiratory centers, the effects of central CO\(_2\) chemodrive were isolated for study. Central chemodrive predominantly influences the magnitude of respiratory muscle force, and thus the peak activity of our neural parameters, while having a lesser effect on timing parameters. To characterize the drive and timing effects in more detail, a subgroup of the phrenic data was analyzed to include two additional parameters of inspiratory drive: SLP (0.1) and PPA/T\(_{1}\), which is the average slope of phrenic activity. T\(_{1}\) and T\(_{8}\) values were also measured. The excellent correlations between PPA and SLP (0.1) and PPA and PPA/T\(_{1}\) confirmed that the relation between \(P_{\text{aco}}\) and

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PPA or peak \( F_t \) concerns mainly central drive and much less \( T_{1} \). Eldridge similarly demonstrated that total phrenic nerve activity (which is dependent on phrenic burst duration) is a relatively poor index of tidal volume or respiratory muscle force output compared with PPA.\(^{20} \)

**Effect of Halothane on Respiratory Timing**

In general, potent inhalational anesthetics in clinically relevant concentrations are thought to increase breathing frequency with increasing dose compared with the awake resting state.\(^{42,43,45-47} \) This response is not abolished or markedly altered by bilateral cervical vagotomy\(^{12,46,47} \) and appears to be predominantly of central origin.\(^{48,49} \) However, at least two studies in cats indicate that halothane can cause a dose-dependent decrease in respiratory rate in a significant number of animals.\(^{50,51} \) Using \( \alpha \)-chloralose–nitrous oxide background anesthesia in cats, Nishino and Honda found that 0.5% halothane produced a small increase in breathing frequency, while 1.0% and 1.5% halothane produced dose-dependent reductions, which were due to increases in \( T_{1} \) that were larger than the accompanying decreases in \( T_{E} \).\(^{50} \) Because a similar respiratory pattern occurred after vagotomy during controlled ventilation, it was concluded that halothane was acting both on the bulbopontine and suprapontine systems.

Our vagotomized dogs showed a dose-dependent decrease in breathing frequency as halothane dose was raised from 1.0 to 2.5 MAC under all \( CO_2 \) drive conditions. This decrease in respiratory rate was accompanied by a moderate dose-dependent decrease in \( T_{1} \) and a larger increase in \( T_{E} \). While increasing \( CO_2 \) drive led to modest increases in \( T_{1} \), no consistent effect of \( CO_2 \) drive could be demonstrated for \( T_{E} \). In the cat, Nishino consistently observed a shortening of \( T_{E} \) with increasing depth of halothane, while there was a progressive increase in \( T_{1} \) for 8 of 12 cats and no change in \( T_{1} \) for the other 4 animals.\(^{51} \) A tendency toward inspiratory apnusis with increasing anesthetic depth is frequently observed in the cat but not in the dog and thus appears to be species specific. At 1.0 MAC halothane, increasing \( CO_2 \) drive resulted in a modest increase in \( T_{E} \) for both cats and our dogs. Under deeper levels of halothane anesthesia the response to \( CO_2 \) resulted consistently in a prolongation of \( T_{E} \) in cats, while the response was variable in our dogs (fig. 7, lower). In summary, the effects of increasing \( CO_2 \) concentration on \( T_{1} \) were directly opposite to those of increasing halothane dose in dogs, but not in cats.

Because we did not study any animals at the 0.5 MAC halothane dose it is possible that we could have demonstrated an increase in respiratory rate between the 0.5 and 1.0 MAC dose as we showed for isoflurane. We routinely observed a transient tachypnea compared with the awake state during mask induction, as well as, a decline in respiratory rate after vagotomy. Increasing \( CO_2 \) drive led to a modest increase in respiratory rate at the deeper MAC levels, while the response was varied at the 1.0 and 1.5 MAC doses. Compared with our isoflurane anesthetized dogs,\(^1 \) respiratory rates at the 1.0 MAC dose halothane were consistently higher at all three \( CO_2 \) drive levels, a result consistent with the human data.\(^{14} \) ANOVA confirmed that the decline in respiratory rate with increasing anesthetic dose was significantly more pronounced with isoflurane than with halothane (\( P \approx 0.0001 \)).

In summary, our studies show that phrenic neural output is more sensitive to the depressant effects of increasing halothane anesthesia than respiratory bulbospinal neuronal activity. The greater depression of phrenic neural output might result from additional anesthetic actions on efferent motor pathways. No significant differential depression of IBS and EBS neuronal activity could be demonstrated. Increases in \( CO_2 \) drive can only partially offset the depressant effect of halothane on central respiratory neural activities.

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