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Effects of Halothane on the Phrenic Nerve Responses to Carbon Dioxide Mediated by Carotid Body Chemoreceptors in Vagotomized Dogs

Eckehard A. E. Stuth, M.D., Zoran Dogas, M.D., Mirko Krolo, M.D.,† John P. Kampine, M.D., Ph.D.,‡ Francis A. Hopp, M.S.,§ Edward J. Zuperku, Ph.D.‖*

Background: **Previous studies in dogs showed that the phrenic nerve response to an acute hypoxic stimulus was dose dependently depressed by 0.5–2.0 minimum alveolar concentration (MAC) of halothane but not abolished. Because a carbon dioxide stimulus is transduced by a different mechanism in the carotid body chemoreceptors (CBCRs) than is a hypoxic stimulus, inhalational anesthetics may preferentially depress one of these transduction processes, the central neuronal processing, or both, of the integrated responses to these two types of inputs.**

Methods: **Carotid body chemoreceptor stimulation was produced by short (1–1.5 s), bilateral, 100% carbon dioxide in saline infusions into the carotid arteries during neural inspiration in unpremedicated, halothane-anesthetized, paralyzed, vagotomized dogs during constant mechanical ventilation. The phrenic neurogram quantified the neural inspiratory response. Four protocols were performed in the study: (1) the dose-dependent effects of halothane anesthesia (0.5–2.0 MAC) during hyperoxic hypercapnia on phrenic nerve activity, (2) the effects of three background levels of the partial pressure of carbon dioxide (Pa_{CO_2}) on the magnitude of the carbon dioxide infusion responses at 1 MAC halothane, (3) the effects of anesthetic type on the magnitude of the carbon dioxide infusion response, and (4) the effects of CBCR denervation.**

Results: **Peak phrenic nerve activity (PPA) increased significantly during the carbon dioxide-stimulated phrenic burst in protocols 1–3; after denervation there was no response (protocol 4). Halothane produced a dose-dependent reduction in the PPA of control and carbon dioxide infusion-stimulated phrenic bursts and in the net carbon dioxide response. The net PPA responses for the different Pa_{CO_2} background levels were not different but were somewhat larger for sodium thiopental anesthesia than for 1.0 MAC halothane.**

Conclusions: **The phrenic nerve response to an acute, severe carbon dioxide stimulus was dose dependently depressed by surgical doses of halothane. The observed responses to carbon dioxide infusion were mediated by the CBCRs because they were eliminated by CBCR denervation. These results suggest that the CBCR transduction and central transmission of the carbon dioxide signal in terms of inspiratory excitatory drive are not abolished at surgical levels of halothane anesthesia.**
(Key words: Anesthetics, volatile: halothane. Ventilation: peripheral ventilatory chemoresponse; carbon dioxide; hypercapnia; normocapnia; hypocapnia. Nerves: phrenic; vagus. Receptors: carotid body chemoreceptors).

THE carotid body chemoreceptors (CBCRs) in mammals are not only responsible for augmenting respiratory output during hypoxia but they also significantly contribute to the increase in ventilation caused by respiratory or metabolic acidosis. The chemoreceptive cells in the mammalian carotid body appear to have two distinct and separate mechanisms for oxygen and acid transduction.[1] Furthermore, these two mechanisms can interact synergistically at the cellular level so that the carbon dioxide response, in terms of the CBCR discharge frequency, is sensitized by hypoxia and the hypoxic response is sensitized by hypercapnia.[1-3] The general consensus is that carbon dioxide activates the CBCR cells exclusively through its intracellular acidifying capacity, whereas other investigators recently hypothesized that oxygen transduction involves an oxygen membrane-binding site (ferroheme proteins) coupled to K+ channels that close when oxygen pressure decreases.[1] Although the latter hypothesis is still controversial, both mechanisms would result in membrane depolarization

* Assistant Professor of Anesthesiology.

† Research Fellow in Anesthesiology.

‡ Professor and Chairman of Anesthesiology.

§ Biomedical Engineer.

‖ Research Professor of Anesthesiology

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Address reprint requests to Dr. Zuperku: Research Service/151, Zablocki VA Medical Center, Milwaukee, WI 53295. Address electronic mail to: ezuperku@mcw.edu

and exocytosis of neurotransmitters that activate the carotid sinus nerve (CSN) afferent fibers.

Halothane might differentially affect these mechanisms. To separate the effects of halothane on the carbon dioxide response of the CBCRs from the hypoxic response, we performed experiments under hyperoxic hypercapnic conditions in unpremedicated, vagotomized dogs using halothane as the only anesthetic. We measured the phrenic nerve response to a brief, near-maximal carbon dioxide stimulus to the CBCRs at four different steady-state halothane doses during hypercapnic hyperoxia. We also examined the interaction between the background level of the partial pressure of carbon dioxide (Pa_{CO_2}), which under hyperoxia is mainly a measure of the central chemodrive inputs, with the CBCR-mediated carbon dioxide bolus response.

Materials and Methods

Surgical Preparation

This study was approved by the Medical College of Wisconsin Animal Care Committee and conformed with standards set forth in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Sixteen adult mongrel dogs (weighing 8–16 kg) were studied under halothane alone (with the exception of protocol 3, explained subsequently). The monitoring and the basic surgical preparation, including bilateral pneumothorax, vagotomy, and the phrenic nerve preparation, were as described previously.[4]

Carotid Body Chemoreceptor Stimulation Technique

To limit the carbon dioxide stimulus to the CBCRs, both common carotid arteries were cannulated *via* the thyroid arteries to deliver brief infusions of 100% carbon dioxide in saline (carbon dioxide pressure $\approx$ 776 mmHg) *via* a pressurized reservoir and solenoid valve system. This technique produces a short-acting, highly reproducible phrenic nerve response that is usually confined to one respiratory cycle without changing the systemic Pa_{CO_2}. The infusion rate was approximately 0.8–1.0 ml/s. Using timing pulses derived from the upstroke of the phrenic neurogram and appropriate circuitry, the triggering time of the solenoid valves was adjusted to the onset of neural inspiration, so that a near-maximal response could be elicited within the same inspiratory phase (fig. 1).

The infusion duration, which produced a near-maximal effect, was determined from the carbon dioxide bolus dose–response curves obtained in the first four animals, in which infusion durations were varied from 0.5–2.5 s at the 1 minimum alveolar concentration (MAC) halothane dose during hypercapnic hyperoxia before protocol 1 was performed (a typical example shown in fig. 2). Durations of 1–1.5 s were used regularly and resulted in infusion volumes of 1–1.5 ml. Once chosen, the same duration was used throughout the experiments in the same animal.

For each experiment the following variables were continuously recorded on a Grass (Quincy, MA) model 7 polygraph: The moving-time average of the efferent phrenic nerve activity from the C5 rootlet phrenic neurogram; carbon dioxide bolus duration; arterial blood pressure; airway carbon dioxide, oxygen, and halothane concentrations; and tracheal pressure.[4]

Protocol 1: Dose-dependent Effects of Halothane on the Phrenic Nerve Response to a Carbon Dioxide Bolus during Hypercapnic Hyperoxia. Eleven animals were studied in protocol 1. 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. A ventilator with an anesthesia circle system without the carbon dioxide absorber was used to ventilate the animals with 100% oxygen at a fixed rate to obtain steady-state hypercapnia (target Pa_{CO_2}, 60–65 mmHg) during hyperoxia (target oxygen pressure [Pa_{O_2}], >400 mmHg). Hyperoxia was used to minimize CBCR activity during the control state. End-tidal halothane, carbon dioxide, and oxygen concentrations were kept constant for at least 15 min before recording the control runs. After recording at least 1 min of control data, a test cycle was initiated by infusing 100% carbon dioxide in saline into both carotid arteries at the onset of a neural inspiration. Test respiratory cycles were repeated three to five times at each anesthetic level, with each test cycle separated by at least 1–2 min of control cycles to allow for complete recovery. These intermittently delivered boli produced no increase in mean Pa_{CO_2} or end-tidal carbon dioxide.

To compensate for possible time-dependent changes in the phrenic neurogram and to allow for comparison with a baseline anesthetic level, the protocol provided for a return to the 1 MAC halothane dose after each

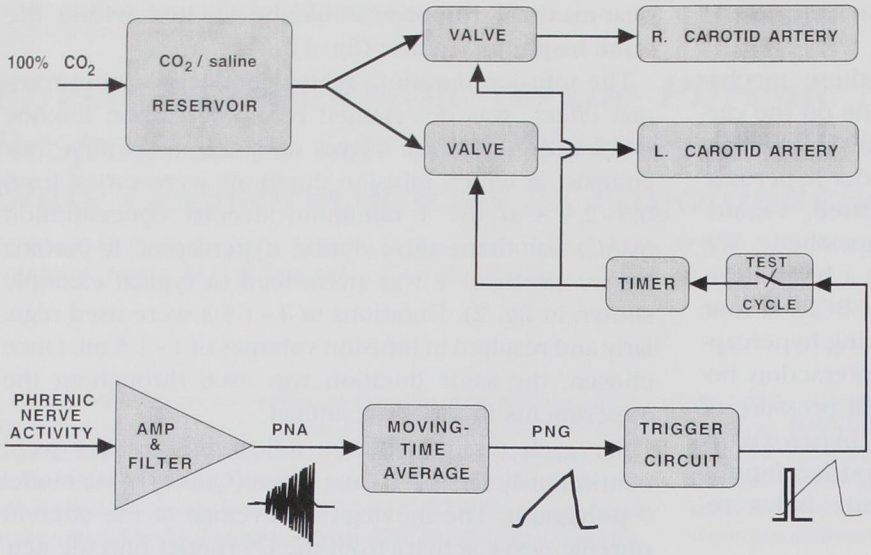

Fig. 1. The apparatus used for the intracarotid carbon dioxide infusions. The onset of the moving time average of the phrenic neurogram (PNG) activates a trigger circuit during the test cycle, which results in the delivery of a short (1–1.5 s) carbon dioxide–saturated saline infusion into the autoperfused common carotid arteries, which stimulates the carotid body chemoreceptors during the same neural respiratory cycle (see the text for details). PNA = phrenic nerve activity.

change in anesthetic depth, as already described for protocol 1 of our companion paper.[4]

Protocol 2: Effects of Background Carbon Dioxide Level on the Phrenic Nerve Response to a Carotid Carbon Dioxide Bolus. In five additional animals that were not part of protocol 1, the magnitude of the phrenic nerve response to the carbon dioxide bolus was studied at three different levels of background carbon dioxide at 1 MAC halothane anesthesia. Experimental conditions were similar to those of protocol 1 except that ventilation was adjusted to produce three levels of Pa_{CO_2}. The low background Pa_{CO_2} level was determined by adjusting tidal volume at a fixed ventilator rate so that Pa_{CO_2} was approximately 2–3 mmHg above the apneic threshold. After completion of the carbon dioxide bolus protocol at the low Pa_{CO_2} target level, dead space was consecutively added to reach the medium Pa_{CO_2} target level (Pa_{CO_2}, 40–45 mmHg), and then the high Pa_{CO_2} target level (Pa_{CO_2}, 60–65 mmHg). Three to five carbon dioxide bolus injections were performed at each background Pa_{CO_2} level.

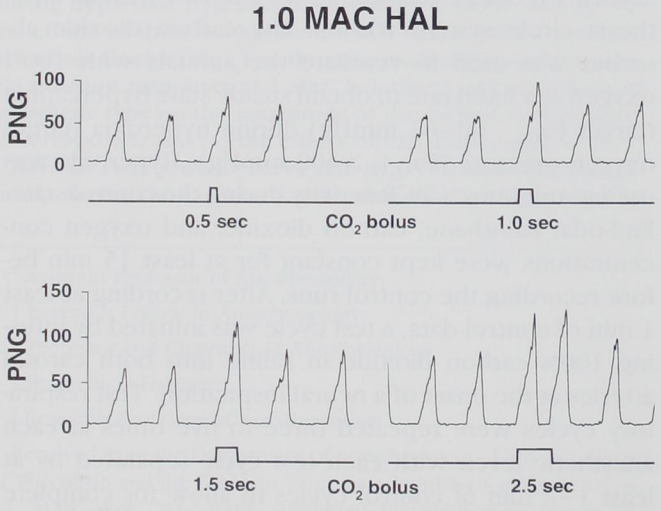

Fig. 2. Example showing the effect of bilateral carbon dioxide–saline infusion duration or bolus size on the magnitude of the peak phrenic neurogram (PNG) at the 1 MAC halothane concentration during hyperoxia. A near-maximal peak response is seen for the 1.5-s duration (carbon dioxide bolus marker). Inspiratory duration (T_I) of the test cycles was also prolonged, whereas the expiratory duration (T_E) was shortened.

Protocol 3: Effects of Anesthetic Type on the Phrenic Response to the Carbon Dioxide Bolus. After completion of protocol 1, we replaced halothane with sodium thiopental (STP) in 3 of the 11 dogs to compare the relative magnitudes of the phrenic nerve response to the carbon dioxide test bolus for these two different anesthetics. A 10 mg/kg bolus of STP was given after completion of protocol 1 and followed by a 4–8 $mg \cdot kg^{-1} \cdot h^{-1}$ continuous intravenous infusion of STP, and then halothane was discontinued. The phrenic responses to the carbon dioxide test bolus were repeated about 90 min after halothane when end-tidal concentrations were less than 0.1%.

Protocol 4: Effect of Carotid Sinus Nerve Denervation on the Phrenic Nerve Response to the Car-

bon Dioxide Bolus. After completion of protocol 2 in all five dogs, the carotid sinus region was exposed, the CSNs were cut, and the carotid bodies were destroyed by crushing and cauterizing the tissue in the sinus region. After this denervation procedure, the anesthetic depth was decreased to the 1 MAC level, and the carbon dioxide bolus response was studied again.

All 16 animals were killed with 4% halothane and a subsequent potassium chloride bolus after data collection.

Data Analysis

Values of peak phrenic nerve activity (PPA), inspiratory duration (T_I), and expiratory duration (T_E) were analyzed off-line by computer at each halothane dose for the two stimulus conditions (hyperoxic control *vs.* carbon dioxide bolus). Averaged data for each respiratory parameter were obtained from test respiratory cycles (n = 3–5) and from the corresponding control cycle that preceded each test cycle. The averaged data for the control cycles during the preceding 1 MAC halothane hyperoxic control condition (protocol 1) and for the 1 MAC hyperoxic hypercapnic control condition for protocols 2, 3, and 4 were used for normalization and assigned a value of 100%. The normalized data were analyzed by applying a two-way analysis of variance technique (SuperAnova; Abacus Concepts, Berkeley, CA) with repeated measures, in which the factors were anesthetic dose and carbon dioxide stimulus state for protocol 1, carbon dioxide background level and carbon dioxide stimulus state for protocol 2, anesthetic agent and carbon dioxide stimulus state for protocol 3, and CSN innervation and carbon dioxide stimulus state for protocol 4, respectively. F-tests were used to determine significant effects of the main factors, and modified *t* tests or the least significant difference method was used to test for significant differences for planned comparisons.[5,6]

For protocol 1, the planned comparisons were to determine whether the carbon dioxide stimulus caused a significant increase in PPA at each anesthetic dose and whether there was a significant dose-dependent depression of the PPA of control and test breath cycles compared with the 1 MAC halothane dose. Planned comparisons for protocol 2 were made for the effect of background carbon dioxide on the net PPA response to the carbon dioxide bolus. Planned comparisons for protocol 3 consisted of testing for differences in the carbon dioxide bolus responses for two types of anesthetic. For protocol 4, planned comparisons were made for the effect of CSN innervation state on the PPA response to the carbon dioxide bolus. Data are presented as mean values with SEs unless otherwise stated. Probability levels of $P < 0.05$ were used to indicate significance.

Results

Protocol 1: Effects of Halothane Dose

Complete protocols were obtained from all 11 animals. The typical 1–1.5 s carbon dioxide infusion produced a brisk increase in PPA and T_I and often shortened the following expiratory phase (T_E) at the 1 MAC halothane baseline level (see fig. 2 [lower], for example).

Figure 3A plots the mean (± SE) normalized PPA as a function of halothane concentration for all 11 dogs. Both the PPA of the control and the test cycles as well as the net PPA response (fig. 3B) showed a dose-dependent decline with increasing halothane dose. In addition, the PPA of the carbon dioxide–stimulated cycles was significantly larger than the PPA of corresponding control cycles at all but the 2 MAC halothane dose.

The 1 MAC end-control values shown were normalized relative to the first 1 MAC control cycle data (rather than to the 1 MAC level preceding the 2 MAC dose) to indicate the relative stability of the phrenic nerve preparation over the entire duration of protocol 1 (typically ≈4 h). The average 1 MAC control value declined from 100% to 77.6 ± 8% ($P < 0.01$) for the final end control, probably because of some time-dependent reduction of the phrenic nerve signal. The phrenic nerve response to the carbon dioxide bolus was consistent for all of the 1 MAC halothane control levels throughout the protocol (*i.e.*, 1.6, 1.54, 1.58, and 2.05 times control).

The average carbon dioxide stimulus–response data for PPA during control hyperoxia and during the test carbon dioxide bolus indicate that increasing the halothane dose produced both a downward shift and a reduction in the average slope or sensitivity of the response data, as indicated by the connecting lines (fig. 3C).

The relative sensitivities of PPA to halothane for control cycles, carbon dioxide stimulation cycles, and net carbon dioxide response were calculated as $S_{rel} = 100$

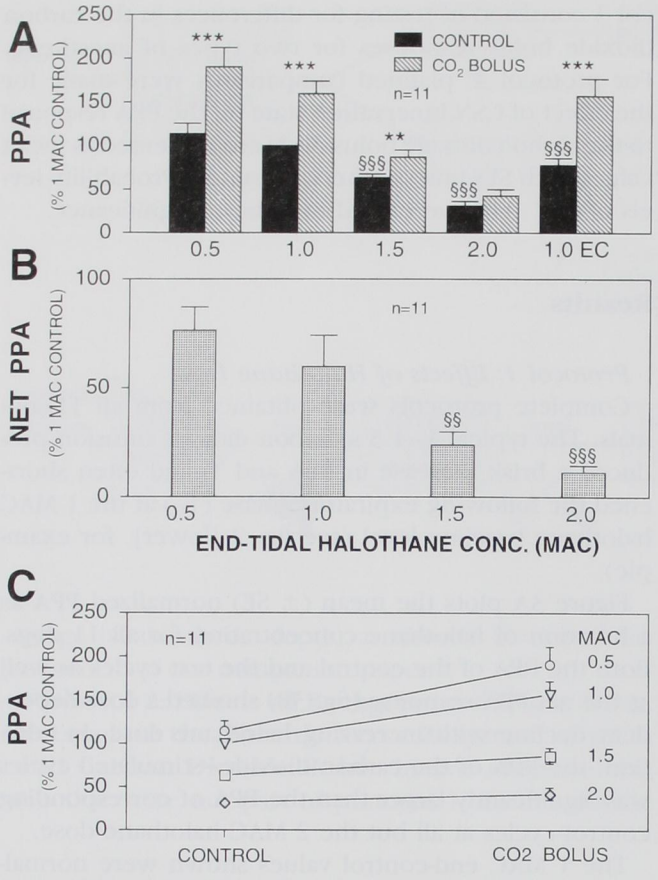

Fig. 3. Effect of halothane dose on peak phrenic nerve activity (PPA) in 11 animals (protocol 1). (*A*) Peak phrenic nerve activity during the hypercapnic hyperoxic control state (*solid bars*) and during the carbon dioxide infusion (*cross-hatched bars*). The PPA values were normalized to the hyperoxic control PPA at 1 MAC. §, Significant differences of the hyperoxic control period PPA values compared with the 1 MAC control level. §§§$P < 0.001$. Asterisks indicate significant increases due to the test carbon dioxide infusions relative to the respective hyperoxic control levels ($P < 0.01$, ***$P < 0.001$). 1.0 EC, 1 MAC end control. (*B*) Dose-dependent depression of the carbon dioxide bolus-induced component of PPA or net PPA response (difference of PPA(bolus) and PPA (control)) by halothane. All net responses were greater than zero. §Difference in net response compared to the 1 MAC dose (§§$P < 0.01$, §§§$P < 0.001$). (*C*) Plots of PPA as a function of the carbon dioxide stimulus for the four halothane doses illustrate that increasing halothane dose reduces the slope (peripheral carbon dioxide sensitivity) and causes a downward baseline shift.**

× [PPA(1 MAC)−PPA(2 MAC)]/PPA(1 MAC) % and indicates the percentage reduction in PPA for a 1 MAC increase. The mean sensitivities were S_{rel}(control PPA), 68.5 ± 4.93%; S_{rel}(CO_2 infusion PPA), 72.8 ± 4.8%; and S_{rel}(net PPA) = 80 ± 5.26%. The halothane sensitivities of both the net carbon dioxide response and the PPA of carbon dioxide infusion cycles were slightly greater than that of the PPA for the control cycles ($P < 0.01$ for both).

Respiratory Phase Durations. The effects of halothane on the normalized inspiratory duration, T_I, and expiratory duration, T_E, for control (*solid bars*) and stimulated (*hatched bars*) cycles are shown in figure 4 (upper and lower, respectively). The average T_I and T_E values, during the first 1 MAC halothane exposure for the hyperoxic control condition, were 1.89 ± 0.17 s and 3.60 ± 0.69 s, respectively. There was a dose-dependent reduction in T_I for both the control and stimulated cycles with increasing halothane dose. In addition, the

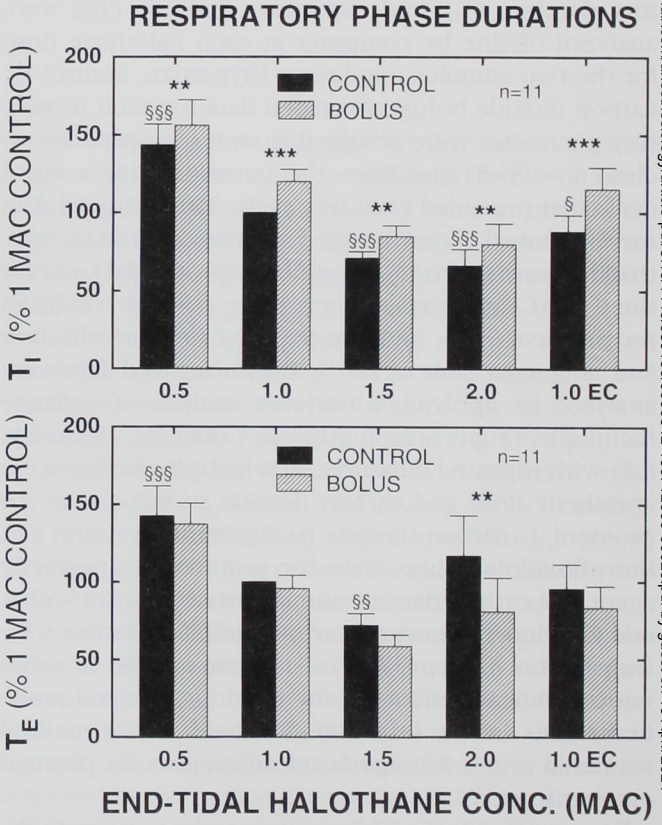

Fig. 4. Effect of halothane dose on inspiratory duration T_I (*upper*) and expiratory duration T_E (*lower*) for control (*solid bars*) and stimulated carbon dioxide bolus breaths (*hatched bars*) in 11 animals (protocol 1). Asterisks indicate significant increases in T_I during the carbon dioxide stimulus and decreases of T_E during the test cycle compared with the respective control cycle ($P < 0.01$; ***$P < 0.001$). §, Significant difference of the control cycle durations compared with the 1 MAC control cycle (§$P < 0.05$, §§$P < 0.01$, §§§$P < 0.001$). 1.0 EC: 1 MAC end-control data obtained at the end of the protocol normalized to the first 1 MAC control values.**

carbon dioxide stimulus consistently caused a small but significant reflex increase in T_I at all doses. Similarly, there was a dose-dependent reduction in T_E with increasing halothane dose; however, at the 2 MAC dose, the overall neural respiratory rate slowed and T_E was prolonged. A consistent but nonsignificant shortening of T_E due to the carbon dioxide bolus effect was observed, except at 2 MAC, where T_E of the test cycles was significantly shorter than for the control cycles. The 1 MAC end control values, which were deliberately normalized to the initial 1 MAC control values, indicate that the respiratory phase durations were relatively stable for the entire duration of protocol 1, but T_I end control values were slightly shorter.

Arterial Blood Gas Data. Because Pa_{CO_2} was not affected by the carbon dioxide bolus infusions during extensive samplings in pilot studies and as also indicated by a lack of change in the end-tidal carbon dioxide concentration, blood gases were sampled only once per anesthetic level in protocol 1. The mean Pa_{CO_2} values were 63.2 ± 1.6 mmHg, 61.1 ± 1.4 mmHg, 63.1 ± 1.4 mmHg, 64 ± 1.3 mmHg, and 63.3 ± 1.4 mmHg for halothane concentrations of 0.5, 1.0, 1.5, 2.0, and 1.0 MAC end control, respectively. These Pa_{CO_2} levels were not significantly different from one another. All animals were ventilated with halothane in 100% oxygen, which ensured Pa_{O_2} levels >400 mmHg.

Protocol 2: Effects of Background Carbon Dioxide Levels

In five additional animals, the effects of three different background Pa_{CO_2} levels on the phrenic nerve response to the carbon dioxide stimulus was studied at the 1 MAC halothane dose. The low, medium, and high background Pa_{CO_2} levels (mean ± SEM) were 36.5 ± 1.1 mmHg, 45.1 ± 1.8 mmHg, and 62.8 ± 1.3 mmHg, respectively. An example from one of these dogs shows that an increase in background Pa_{CO_2} from 36.5 to 63 mmHg produced a threefold increase in hyperoxic PPA (fig. 5A, left). The carbon dioxide bolus caused a large increase in PPA and T_I of the test cycle at all three background Pa_{CO_2} levels. T_E of the test cycle was also shortened. Figure 5B shows the pooled data of all five dogs. The calculated net phrenic responses to the carbon dioxide bolus (normalized to the control PPA at high carbon dioxide) for the low, medium, and high Pa_{CO_2} levels were 61 ± 37.4%, 75 ± 27.5%, and 60 ± 12.4%, respectively. Statistical analysis indicates no significant difference in these net responses for the three background Pa_{CO_2} levels.

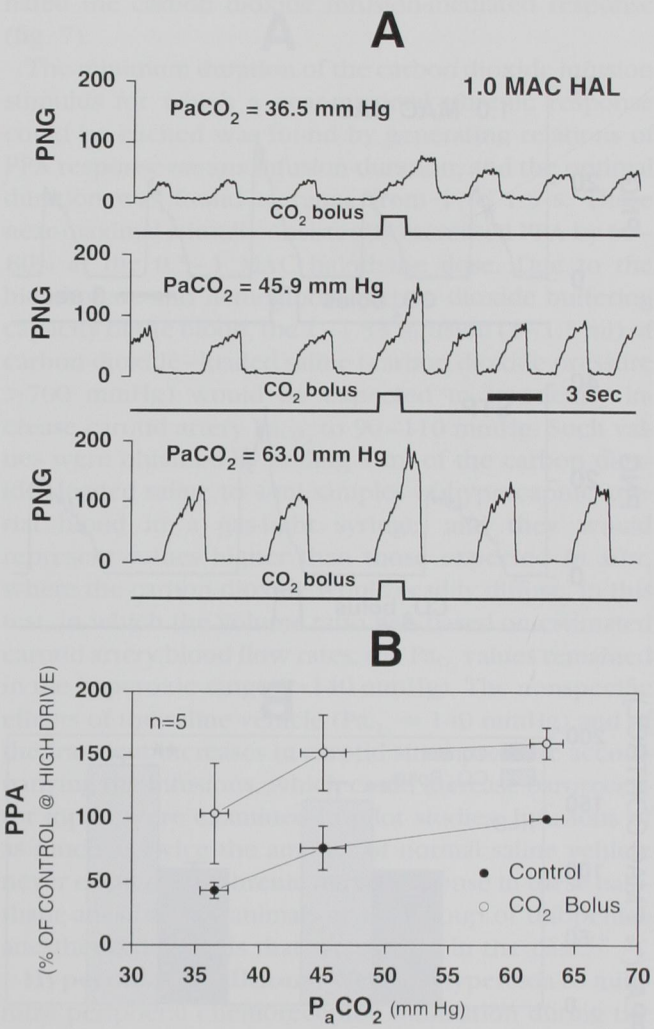

Fig. 5. (*A*) Effect of three levels of background Pa_{CO_2} on phrenic nerve activity (PNG) during the hyperoxic control and carbon dioxide stimulation cycles (carbon dioxide bolus marker) at 1 MAC halothane. (*B*) Plot of peak phrenic nerve activity (PPA) *versus* Pa_{CO_2} (n = 5; protocol 2). *Filled circles:* hyperoxic control PPA; *open circles:* PPA of the carbon dioxide bolus-stimulated breaths. There were no significant differences in the net phrenic responses to the carbon dioxide bolus for the three Pa_{CO_2} levels.

Protocol 3: Effects of Anesthetic Type

After completion of protocol 1 in 3 of the 11 dogs, halothane was discontinued and replaced by STP. Carbon dioxide bolus responses were then obtained 90 min after halothane. An example from one of the animals

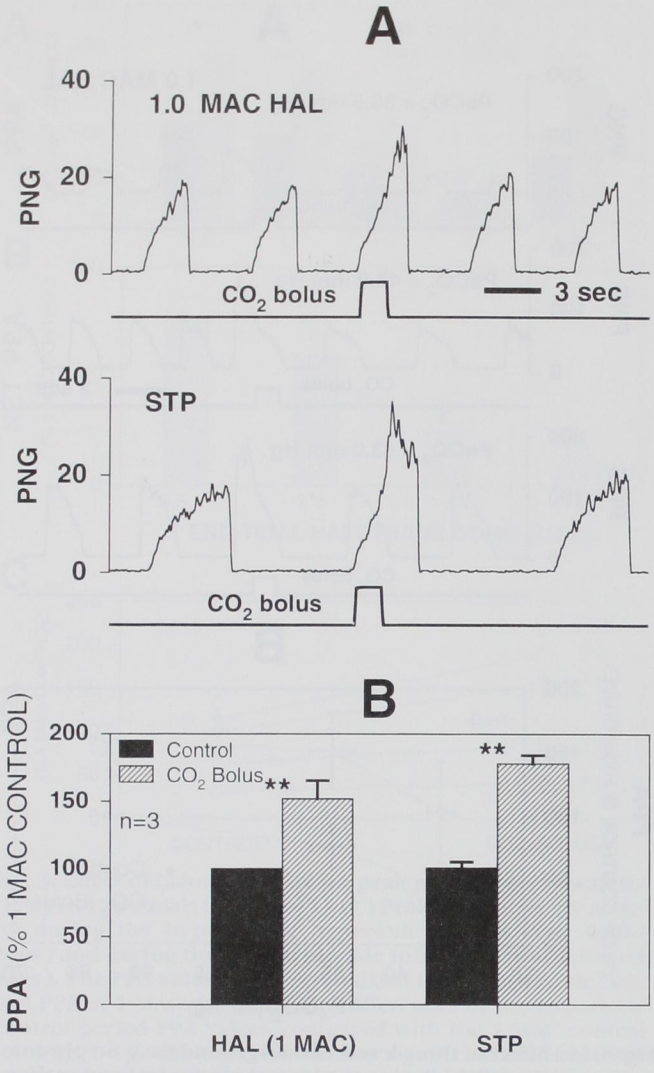

Fig. 6. (*A*) Typical example of the effect of a 1.5-s carbon dioxide bolus (marker) on phrenic nerve activity (PNG) during 1 MAC halothane (HAL) anesthesia and 90 min after replacement of halothane with intravenous sodium thiopental (STP) anesthesia. The two anesthetics have strikingly different effects on neural phase timing, but the carbon dioxide bolus caused a comparable increase in peak phrenic activity with both anesthetics. (*B*) Pooled peak phrenic activity (PPA) data of the three animals subjected to the two different anesthetics (protocol 3). The carbon dioxide bolus caused a significant increase in PPA with both types of anesthetics ($P < 0.01$), but the net response during STP was greater than that during halothane ($P < 0.05$).**

indicates that the magnitude of the carbon dioxide bolus response at the 1 MAC halothane level was comparable to that obtained during STP (fig. 6A). It is also apparent that the neural respiratory rates and breathing pattern differ between these two agents. The pooled data from three animals indicate that the carbon dioxide bolus caused a significant increase in PPA for both agents. The net response during STP (78 ± 6.1%) was larger than during 1 MAC halothane (51.9 ± 13.7%; $P < 0.05$).

Protocol 4: Carotid Sinus Denervation

Figure 7 shows a representative example of the effect of bilateral carotid body denervation at the 1 MAC halothane dose. Before denervation, the carbon dioxide bolus nearly doubled the PPA of the test cycle compared with control and prolonged T_I while it shortened the T_E of the test cycle. After denervation (CSN cut), the carbon dioxide bolus caused no change in these parameters. The mean carbon dioxide bolus-induced increase in PPA (100% to 181.9 ± 9.8%) was completely abolished by CSN denervation (100% *vs.* 103.3 ± 2%) in all five dogs.

Discussion

The main findings of this study indicate that the phrenic nerve response to carbon dioxide, mediated by the

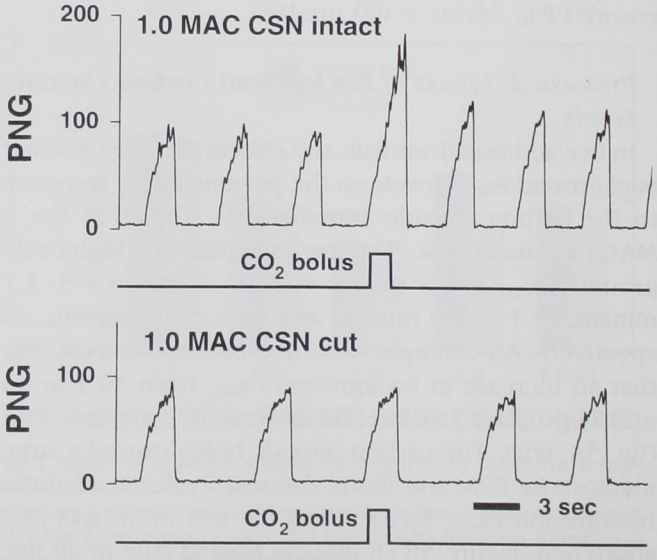

Fig. 7. Typical example taken from one of the five dogs illustrating the effect of carotid body chemoreceptor denervation on the carbon dioxide bolus-induced responses of phrenic nerve activity (PNG) at 1 MAC halothane. The increase in PNG caused by the carbon dioxide bolus and its effects on phase timing are completely abolished after denervation. CSN = carotid sinus nerve.

CBCRs, is clearly present at surgical levels of halothane anesthesia. The net carbon dioxide bolus-mediated response and the PPA of control cycles were dose dependently depressed over the dose range from 0.5 to 2 MAC. A small but nonsignificant carbon dioxide response could still be observed at the 2 MAC level (*i.e.*, 19% of the control net response at 1 MAC). The carbon dioxide bolus infusion technique produced average increases of 60–70% in PPA at the 1 MAC dose. Although protocol 1 studies were performed during isocapnic hypercapnia (Pa_{CO_2}, 61–64 mmHg) to ensure rhythmic phrenic activity at 2 MAC halothane, the magnitude of the net carbon dioxide response was similar for the hypo- to normocapnic range at the 1 MAC level (fig. 5B). Thus during hypercapnia, the magnitude of the carbon dioxide bolus-induced response and the effects of halothane on it do not appear to be either under- or overestimated secondary to the increase in overall carbon dioxide drive, and they may be considered representative of those expected during normocapnia.

Critique of Methods

Open-loop Conditions. The main focus of this study was analyze the dose-dependent effects of halothane on the transmission of carbon dioxide–evoked, CBCR-mediated excitation to the phrenic motoneurons. To avoid confounding effects, many of the factors that can alter phrenic nerve activity were controlled or eliminated, as discussed in our companion article in this issue of ANESTHESIOLOGY.[4]

Carbon Dioxide Stimulus. The use of short duration, bilateral infusions of carbon dioxide–saturated saline into the autoperfused common carotid arteries, with the tip of the infusion catheters positioned 1–2 cm proximal to the carotid sinus, allowed a near-maximal, simultaneous bilateral stimulation of the CBCRs within and largely limited to the same neural respiratory cycle. The rapid onset (<100 ms) and brief duration of the response leave no doubt about the selective nature of the stimulus. Furthermore, the lack of a change in overall mean Pa_{CO_2} or end-tidal carbon dioxide, and the fact that the boli were injected into the carotid arteries rather than into the vertebral arteries that supply the brain stem, make it highly unlikely that the carbon dioxide boli could reach the central chemoreceptors, which are thought to be located near the ventral surface of the medulla and in deeper structures of the brain stem.[7] In addition, bilateral CSN transection completely eliminated the carbon dioxide infusion-mediated response (fig. 7).

The minimum duration of the carbon dioxide infusion stimulus for which a near-maximal phrenic response could be elicited was found by generating relations of PPA response *versus* infusion duration, and the optimal duration was found to range from 1 to 1.5 s. These near-maximal stimuli consistently increased PPA by 60–80% at the 0.5–1 MAC halothane dose. Due to the bicarbonate and hemoglobin carbon dioxide buffering capacity of the blood, the 1–1.5 s infusion (1–1.5 ml) of carbon dioxide–loaded saline (carbon dioxide pressure >700 mmHg) would be expected to transiently increase carotid artery Pa_{CO_2} to 90–110 mmHg. Such values were obtained by adding 1 ml of the carbon dioxide–loaded saline to 4-ml samples of hypercapnic arterial blood in a gas-tight syringe, and they would represent values higher than those expected *in situ*, where the carbon dioxide would readily diffuse. In this test, in which the volume ratio was based on estimated carotid artery blood flow rates, the Pa_{O_2} values remained in the hyperoxic range (>140 mmHg). The nonspecific effects of the saline vehicle ($Pa_{O_2} \approx 140$ mmHg) and of the transient increases in carotid sinus pressure accompanying the infusions, which could increase baroreceptor input, were examined in pilot studies. Infusions of as much as twice the amount of normal saline vehicle never elicited any phrenic nerve response in these halothane-anesthetized animals or in a group of thiopental-anesthetized animals that we studied in the past.[8,9]

Hyperoxic Conditions. We used hyperoxia to minimize peripheral chemoreceptor stimulation during the control state. At a Pa_{O_2} >400 mmHg, the slope of the carbon dioxide response curve of the CBCRs is small, and our hypercapnic background Pa_{CO_2} level would be expected to contribute only a small fraction of the excitatory drive to the phrenic nerve output *via* the CBCRs. Various studies suggest that at such high levels of hyperoxia the peripheral chemoreceptors contribute only 13–14% to the overall carbon dioxide sensitivity.[10,11] Because the carbon dioxide bolus produced transient increases of about 40 mmHg in carotid artery carbon dioxide pressure and 60% in PPA, the sensitivity due to the CBCRs appears to be similar to that due to the central chemosensors, as estimated from the data of figure 5B. However, because of the carbon dioxide rate sensitivity of the CBCRs, the peak response to a step increase in Pa_{CO_2} is three to four times the steady-state response.[12] Taking this into consideration, our esti-

mated peripheral carbon dioxide sensitivity would be 15–18% of the overall carbon dioxide sensitivity, which corresponds well with that of others.[10,11] Thus, during the control state, phrenic nerve activity appears to dependent primarily on central chemosensory excitation, whereas the net carbon dioxide bolus response is due to CBCR-mediated excitation. Furthermore, the data of protocol 2 suggest that the net PPA response is relatively independent of the level of central carbon dioxide drive and that the two drives are approximately additive (fig. 5B; parallel upward shift).

Differential Sensitivities to Halothane. The relative sensitivity of the net carbon dioxide response (net PPA) to a 1 MAC change (80% decrease at 1 MAC) was slightly greater than that for control cycles (68.5% decrease at 1 MAC). This finding is also consistent with the slightly larger responses obtained during STP anesthesia 90 min after discontinuation of halothane in 3 of the 11 dogs (fig. 6). This suggests that there might be a slightly greater depression of the CBCRs and associated neural pathways to the phrenic motoneurons compared with those for the central chemosensors and associated neural pathways. The sensitivity to halothane may include both a reduction of carbon dioxide sensitivity and neural transmission and a downward shift in baseline activity. Similar data for the acute hypoxic stimulation studies indicated no significant differences in the PPA sensitivities (S_{rel}, 60–64%) to halothane for the hypoxic and control states.[4] A possible explanation for these minor differences may be the magnitude of the CBCR stimulation secondary to a different severity of the two stimuli. The average net increase in PPA due to hypoxia was 94.4% of control, whereas that for the carbon dioxide infusion was 59.6% of control at the 1 MAC halothane level. This observation seems analogous to that of Ponte and Sadler,[13] who found that the depressant effect of halothane on CBCR unit activity was less during severe compared with milder levels of hypoxia. Our overall findings of a similar depression of the CBCRs and associated pathways and central chemosensors and associated pathways by halothane are consistent with those of the Leiden respiratory group, which used a feline artificial brain-stem perfusion model[14,15] and found that peripheral and central carbon dioxide sensitivities (in terms of minute ventilation) were similarly depressed by halothane.

The present studies include the dose-dependent depressant effects of halothane on the CBCR transduction of the carbon dioxide stimulus and the intervening central neuronal pathways to and including the phrenic motoneurons. In this regard we previously found that medullary inspiratory bulbospinal neurons are also dose-dependently depressed by halothane.[16] A 1 MAC increase in halothane (1–2 MAC) during hypercapnia ($Pa_{CO_2} \approx 67$ mmHg) produced a 46% decrease in peak inspiratory bulbospinal neuronal discharge frequency and a 70% decrease in PPA. These findings suggest that brain-stem pathways are significantly depressed by halothane and that halothane has a significant depressant effect on the phrenic motoneurons themselves. Our studies do not allow us to assess the degree of depression of the CBCRs *per se*. However, the recordings from CBCR fibers in rabbits and cats by Ponte and Sadler[13] indicated that 0.5–1% halothane had no effect on the carbon dioxide response curves, leading these investigators to suggest that the depression of the hypercapnic ventilatory response in the dog reported by others[17,18] may be due to a central effect of halothane rather than to an effect on the CBCRs.

In summary, our studies show that halothane produces a dose-dependent depression of the phrenic nerve response to carbon dioxide stimulation of the CBCRs, but these responses were not abolished at surgical levels of halothane, suggesting that the CBCRs remain responsive to carbon dioxide in dogs under halothane anesthesia.

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