Enflurane Depresses Activity of the Medullary Inspiratory Neurons in the Cat (43122)

MAHMOOD TABATABAI, LUKE M. KITAHATA, MICHIO KAWAHARA, AND J. G. COLLINS

Department of Anesthesiology, Yale University School of Medicine, New Haven, Connecticut 06510, and Department of Anesthesiology, University of Pittsburgh School of Medicine, and Veterans Administration Medical Center, Pittsburgh, Pennsylvania 15240

Abstract. **The effect of enflurane on the firing activity (spikes/sec) of the inspiratory neurons of the dorsal respiratory group (DRG) of the medulla oblongata was studied in decerebrate, paralyzed, mechanically ventilated cats before and after bilateral cervical vagotomy. Inspiratory neuronal activity, phrenic neurogram, arterial blood pressure, tracheal pressure, and end tidal CO_2 concentration were recorded. Cells whose firing activity was in phase with that of the phrenic nerve were considered inspiratory neurons. Administration of 1 and 2% enflurane in oxygen produced gradual, significant, and dose-dependent depression of the cell activity with cervical vagi either intact or severed. Recovery of the cell activity occurred after termination of enflurane administration. In cats with intact vagi, 10 min after introduction of 1 and 2% enflurane, the cell activity (mean ± SE) expressed as percentage of the control was 70 ± 6% ($P < 0.05$) and 48 ± 5% ($P < 0.01$), respectively. Bilateral cervical vagotomy did not affect the degree of cell depression due to enflurane. Hypercarbia induced by inhalation of 5% CO_2 increased cell activity, but it did not block enflurane-induced cell depression, although it reduced it. It may be concluded that enflurane depresses the activity of the inspiratory neurons of the DRG. The results also suggest that the respiratory depressant effect of enflurane has a central component and that the DRG region may serve as a site to mediate the enflurane-induced respiratory depression.** [P.S.E.B.M. 1990, Vol 195]

Inhalational anesthetics (such as halothane, enflurane, and isoflurane) depress respiration (1–5) and decrease the ventilatory response to hypercarbia (6, 7). It is generally assumed that the respiratory depression induced by inhalational anesthetics is due to the action of these agents on brainstem respiratory neurons. To test this assumption, the present study was designed to determine the effects of enflurane, a halogenated inhalational anesthetic, on the medullary inspiratory neurons in decerebrate cats before and after bilateral cervical vagotomy.

Materials and Methods

All institutional, state, and federal guidelines for the use of research animals were followed carefully during all aspects of this study. Seven cats weighing 2.5–3.5 kg were anesthetized with a mixture of halothane, nitrous oxide, and oxygen. The trachea and a femoral artery and vein were cannulated. The animals were ventilated mechanically with the anesthetic mixture using a Harvard ventilator and paralyzed with intravenous gallamine. Additional doses of gallamine were given at hourly intervals to maintain muscle paralysis. The cervical vagi were isolated and kept moist by application of 0.9% sodium chloride solution. The cats were then fixed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) and rendered decerebrate according to a previously described method (8). After decerebration, halothane and nitrous oxide were turned off, whereas oxygen was continued. 100% oxygen was used to minimize the activity of the peripheral chemoreceptors (9). Posterior fossa craniotomy was done to expose the medulla oblongata. The C_5 branch of the phrenic nerve was exposed, desheathed, and kept in a pool of paraffin to prevent drying of the nerve. Details of surgical procedures have been given elsewhere (10).

We used bipolar silver wire electrodes to record the

Received July 25, 1989. [P.S.E.B.M. 1990, Vol 195]
Accepted April 25, 1990.

0037-9727/90/1951-0079$2.00/0
Copyright © 1990 by the Society for Experimental Biology and Medicine

phrenic neurogram on a cathode rat oscilloscope (Tektronix, Beaverton, OR) and on a polygraph (Grass Instrument Co., Quincy, MA). We inserted a tungsten microelectrode (Frederick Haer and Co., Brunswick, ME), with a tip diameter of 1 μm and an impedance of 10 MΩ, stereotaxically into the dorsal respiratory group (DRG) in the region of the nucleus of the tractus solitarius with the aid of a hydraulic microdrive (David Kopf Instruments). The microelectrode signal was amplified, passed through an amplitude discriminator (Quasitronics, Inc., Pittsburgh, PA), and monitored on the cathode rat oscilloscope. Any cell whose periodic firing activity was in phase with that of the phrenic nerve was considered an inspiratory neuron (Fig. 1). Relative to the obex, the coordinates of the area where inspiratory neurons were found by microelectrode insertion were between the obex and 2 mm rostral to the obex, from 2 to 3 mm lateral to the obex, and at a depth of 2–2.5 mm from the posterior surface of the medulla oblongata. Furthermore, the output from the amplitude discriminator was passed through a rate meter and recorded on the polygraph as impulses per sec (Fig. 2), showing instantaneous peak firing frequency of the cell. The femoral arterial blood pressure and tracheal pressure were recorded on the polygraph. Thus, continuous recording of the activity of the inspiratory neuron, phrenic neurogram, arterial blood pressure, and tracheal pressure were made on the polygraph, whereas the inspiratory neuronal activity and phrenic neurogram were displayed simultaneously on the oscilloscope as well (Fig. 1). (The tracheal pressure was measured via a polyethylene tube (PE-200), inserted into the lumen of the tracheal cannula through a hole made on the side of the cannula. The polyethylene tube was advanced until its tip was in the distal third of the cannula. Then, the side hole was sealed with glue. The peak inspiratory tracheal pressure during mechanical ventilation varied from 4 to 6 mm Hg.) Whenever the systolic arterial blood pressure dropped below 100 mm Hg during administration of enflurane (two cats), the pressure was raised to 100 mm Hg by intravenous infusion of epinephrine drip. Rectal temperature was monitored by a thermistor (Yellow Springs Instrument Co., Yellow Springs, OH) and kept at 37 ± 1°C by means of thermostatically controlled heating devices. End tidal CO_2 was measured by an infrared gas analyzer (Beckman Instruments) and maintained at 4%.

Enflurane was administered using an anesthesia machine and a calibrated vaporizer (Ohio Medical Products, Madison, WI). The oxygen flow was kept constant at 2 liters/min throughout the experiment. The oxygen or the enflurane-oxygen mixture was introduced into the inspiratory port of the respirator. The time between termination of halothane-nitrous oxide and beginning of enflurane administration was about 3 hr. The inspired concentrations of enflurane administered before bilateral cervical vagotomy were 1 and 2%. Sufficient time was allowed to pass between administration of 1 and 2% enflurance, such that the cells' activity (spikes/sec) might return to control value and remain so for at least 15 min. After bilateral cervical vagotomy, enflurane was delivered in the same sequence of 1 and 2% with time in between for full recovery of the cell activity. The cells' activity as percent of control was compared with the control value (pre-enflurane value). Repeated measures analysis of variance and Neuman-Keuls test where used to analyze the data. P values < 0.05 were considered statistically significant.

To show the effect of hypercarbia on the response of the cells to enflurane, two of the cats were given 5% CO_2 in oxygen for 5 min. At the end of the 5-min period, enflurane-CO_2-O_2 mixture was introduced for

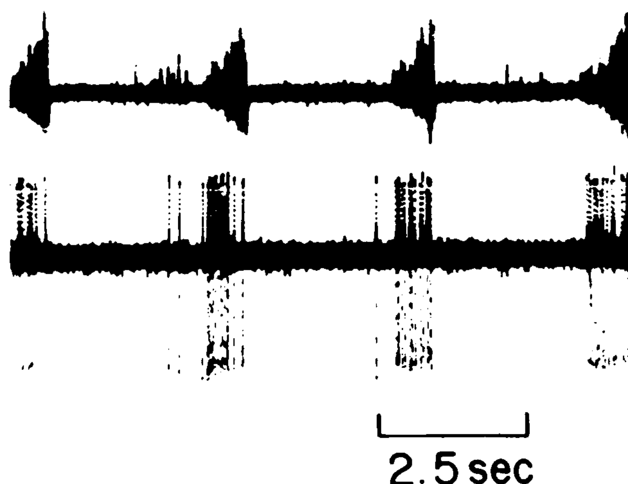

Figure 1. Oscilloscopic recording of phrenic neurogram (upper trace) and an inspiratory neuron firing activity (lower trace). The rhythmic activity of the inspriatory neuron is synchronous with that of the phrenic nerve. The inspiratory neuron belongs to the dorsal respiratory group of the medulla oblongata.

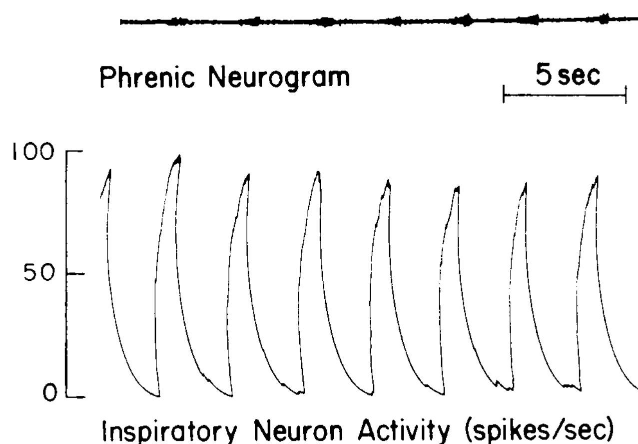

Figure 2. Polygraph recording of phrenic neurogram (upper trace) and the instantaneous peak firing activity (spikes/sec) of an inspiratory neuron of the dorsal respiratory group of the medulla oblongata (lower trace).

10 min. This procedure was carried out before and after vagotomy.

Results

We were able to maintain and study seven cells for 10 min in seven cats (one cell in each cat), and five cells for 30 min in five cats (five of the seven cats) during administration of 1% enflurane before bilateral cervical vagotomy. For ease of comparison, an equal number of cells, i.e., seven cells for 10 min and five cells for 30 min, were studied during administration of 2% enflurane. Similarly, after bilateral cervical vagotomy, we studied seven cells for 10 min and five cells for 5 min during administration of 1 and 2% enflurane. Enflurane depressed activity of the cells (spikes/sec) in a gradual fashion. The magnitude of the depression was dependent on the duration of exposure to and the concentration of the anesthetic (Fig. 3). The cell depression was reversible, and the neuronal activity gradually returned to the control level on termination of enflurane administration (Fig. 3).

With 1% enflurane, the degree of cell depression was significant ($P < 0.05$) at 10 min and thereafter. The level of cell activity (spikes/sec) as percentage of control at 5 and 10 min after enflurane introduction was 85 ± 7% (mean ± SE) and 70 ± 6% (mean ± SE), respectively, before bilateral cervical vagotomy.

With 2% enflurane, the degree of cell depression was significant at 5 min and thereafter. The level of cell activity as percentage of control at 5 and 10 min after enflurane introduction was 72 ± 5% (mean ± SE) ($P < 0.05$) and 48 ± 5% ($P < 0.01$), respectively.

Bilateral cervical vagotomy had no effect on the cell response to enflurane administration; the difference in the amount of cell depression with vagi intact and severed was insignificant.

Hypercarbia, induced in two cats by administration of 5% CO_2 in oxygen and evidenced by a rise in the end tidal CO_2 to 7.5–8% after 5 min of inhalation, increased the peak firing activity of the inspiratory neurons by 35%. However, hypercarbia did not block the depressant effects of enflurane on the inspiratory neurons. Ten minutes after inhalation of 2% enflurane in O_2-CO_2 mixture, the cell activity (spikes/sec) was 55% of control (and replace with), in contrast to 48% when 2% enflurane was administered in oxygen alone.

Discussion

The results of this study show that enflurane significantly depresses the firing activity of the medullary inspiratory neurons in a gradual and dose-dependent manner and that the depression is a reversible process; on termination of enflurane administration, the activity gradually returns to control value. Enflurane-induced inspiratory neuronal depression occurs in the eucarbic and hypercarbic animals with the cervical vagi either intact or severed.

The results also suggest that the respiratory depressant effect of enflurane has a central component and that a site of action of enflurane is the DRG of the medulla in the region of the nucleus of the tractus solitarius. This conclusion is based on the fact that the cells studied were in the DRG region. However, enflurane could conceivably have affected any of the brainstem respiratory neurons. There are three groups of brainstem respiratory neurons, one in the pons and two

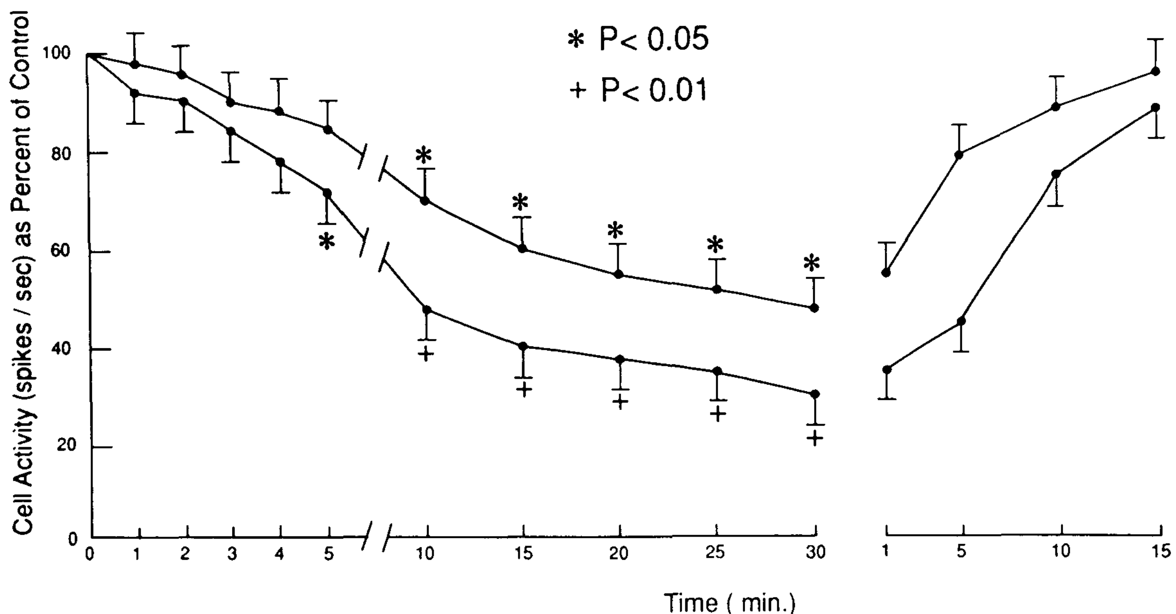

Figure 3. Effect of administration of 1% (upper curve) and 2% (lower curve) enflurane on the activity of the inspiratory neurons of the dorsal respiratory group of the medulla after bilateral cervical vagotomy. Each point represents mean ± SE. Seven cells were studied during the first 10 min, and five cells during the last 20 min. Recovery of the activity of the 5 cells is shown on the right panel starting at 1 min after enflurane discontinuation up to 15 min.

in the medulla oblongata (11). Of the two in the medulla, one group, composed primarily of inspiratory neurons and closely associated with the nucleus of the tractus solitarius, lies in the dorsomedial part of the medulla and is termed dorsal respiratory group (12–14). The second one, composed of both inspiratory and expiratory cells, is located in the ventrolateral part of the medulla and is termed the ventral respiratory group (15, 16). The pontine respiratory neurons are located in the rostral pons and referred to as pontine respiratory group or pneumotaxic center (16).

Whereas the spinal cord respiratory motoneurons receive their input from the medullary respiratory neurons, not all of the latter neurons project to the spinal cord respiratory motoneurons. For example, within the DRG, on the basis of response to lung inflation, three types of cells have been recognized. One type called I α (Inspiratory α) (17) or R α (Respiratory α) (18) is inhibited by lung inflation. The axons of these cells project both to the phrenic and external intercostal motoneurons of the spinal cord (15). Another type, called I β (17) or R β (18) is excited by lung inflation. There is controversy as to whether the I β axons project into the spinal cord respiratory motoneurons. von Euler (15), citing the recent unpublished observations of Kalia and Richter, states that some of the I β cells project into the spinal cord, although the functional significance of these spinal projections has yet to be determined. Both I α and I β cells receive excitatory inputs from the respiratory central pattern generator (17, 19), such that when the lung inflation is stopped, or the vagus nerves are cut in the neck, the discharge activity of these neurons would continue. There are also a number of cells in the DRG without input from the respiratory central pattern generator. The discharge activity of these cells depends on and follows lung inflation during either spontaneous or controlled ventilation; these are called pump or "P" cells and are assumed to be interneurons (17).

The cells we studied could not have been "P" cells, because their rhythmic firing activity continued in the absence of input from lung inflation after bilateral cervical vagotomy. However, we cannot say whether they were I α or I β type cells.

Since the degree of cell depression was the same before and after bilateral cervical vagotomy, the depressant effect of enflurane on the DRG neurons is independent of its actions on the pulmonary system. And since the cats were being ventilated with 100% oxygen before enflurane introduction, the peripheral chemoreceptors should have had little activity in the presence of hyperoxia (9), and therefore minimal excitatory impulses to the brainstem respiratory neurons. Consequently, the depression of the DRG neurons could not be attributed to enflurane-induced cessation of excitatory impulses from peripheral chemoreceptors to the brainstem.

As a conceivable explanation for the decreased tidal volume and minute ventilation during enflurane anesthesia, on the basis of our results, it may be said that decreased activity of the medullary inspiratory neurons will lead to decreased efferent inspiratory impulses from the medulla to the spinal respiratory motoneurons, and consequently to the inspiratory muscles (external intercostal and diaphragm muscles). As a result, the inspiratory muscles would not contract adequately, causing decreased tidal volume and minute ventilation.

Based on the results of the present study, it may be concluded that enflurane depresses the activity of the inspiratory neurons of the DRG. The results also suggest that the respiratory depressant effect of enflurane has a central component and that the DRG region may serve as a site to mediate the enflurane-induced respiratory depression.

This work was supported by National Institutes of Health Grant NS-09871.

We wish to thank Michelle Poropatic for her secretarial assistance.

1. Munson ES, Larson CP, Babad AA, Regan MJ, Buichel DR, Eger EI. The effects of halothane, fluroxene, and cyclopropane on ventilation: A comparative study in man. Anesthesiology **27**:716–728, 1966.
2. Larson CP, Eger EI II, Muallem M, Buichel DR, Munson ES, Eisele JH. The effects of diethyl ether and methoxyflurane on ventilation. II. A comparative study in man. Anesthesiology **30**:174–184, 1969.
3. Nagi SH, Mark LC, Papper EM. Pharmacologic and physicologic aspects of anesthesiology. N Engl J Med **282**:541–557, 1970.
4. Fourcade HE, Stevens WC, Larson CP, Cromwell TH, Bahlman SH, Hickey RF, Halsey MJ, Eger EI. The ventilatory effects of forane, a new inhaled anesthetic. Anesthesiology **35**:26–31, 1971.
5. Calverly RK, Smith NT, Jones CW, Eger EI. Man partially adapts to the ventilatory depressant effects of enflurane anesthesia. Abstracts of Scientific Papers, ASA Annual Meeting, p259, 1975.
6. Knill RL, Gelb AW. Ventilatory response to hypoxia and hypercapnia during halothane sedation and anesthesia in man. Anesthesiology **49**:244–251, 1978.
7. Knill RL, Mannimen P, Clement JL. Ventilation and chemoreflexes during enflurane sedation and anesthesia in man. Can Anaesth Soc J **26**:353–360, 1979.
8. Kitahata LM, Losaka Y, Taub A, Bonikos K, Hoffert M. Lamina-specific suppression of dorsal-horn unit activity by morphine sulfate. Anesthesiology **41**:39–48, 1974.
9. Biscoe TJ, Purves MJ, Sampson SR. The frequency of nerve impulses in single carotid body chemoreceptor afferent fibers recorded in vivo with intact circulation. J Physiol (Lond) **208**:121–131, 1970.
10. Tabatabai M. Respiratory and cardiovascular responses resulting from heating of the medulla oblongata in cats. Am J Physiol **222**:1558–1564, 1972.
11. Tabatabai M, Vazir H, Howard BR. Central respiratory mechanisms. Pahlavi Med J **5**:496–531, 1974.
12. von Baumgarten R, von Baumgarten A, Schaefer KP. Beitrag

zur lokalisationsfrage bulboreticularer respiratorischer neuron der katze. Pfluegers Arch Gesamte Physiol Menschen Tiere **264**:217–227, 1957.

13. Berger AJ. Dorsal respiratory neurons in the medulla of cat: Spinal projections, responses to lung inflation and superior laryngeal nerve stimulation. Brain Res **135**:231–254, 1977.
14. von Euler C, Hayward JN, Martilla I, Wyman RJ. Respiratory neurons of the ventrolateral nucleus of the solitary tract of cat. Vagal input, signal connections and morphological indentification. Brain Res **61**:1–22, 1973.
15. von Euler C. Brain stem mechanisms for generation and control of breathing pattern. In: Cherniack NS, Widdicome JC, Eds. Handbook of Physiology. Bethesda, MD: American Physiological Society, Vol **II**: pp1–67, 1986.
16. Feldman JL. Neurophysiology of breathing in mammals. In: Bloom FE, Ed. Handbook of Physiology. Bethesda, MD: American Physiological Society, Vol **IV**: pp463–524, 1986.
17. Cohen MI. How is respiratory rhythm generated? Fed Proc **40**:2372–2377, 1981.
18. von Baumgarten R, Kanzow E. The interaction of two types of inspiratory neurons in the region of tractus solitarius of the cat. Arch Ital Biol **96**:361–373, 1958.
19. Feldman JL, Speck DF. Interaction among inspiratory neurons in dorsal and ventral respiratory groups in cat medulla. J Neurophysiol **49**:472–490, 1983.