Effects of Halothane on Medullary Inspiratory Neurons of the Cat

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The effect of halothane on the electrical activity of inspiratory neurons of the nucleus tractus solitarius (NTS) was studied in decerebrate, paralyzed, mechanically ventilated cats. Simultaneous recording of the activity of the neurons of the NTS and the phrenic nerve was done to identify the inspiratory neurons. Cells whose firing activity was synchronous with that of the phrenic nerve were considered inspiratory neurons. Administration of 1% and 1.5% halothane in oxygen induced a dose-dependent depression of the cell activity (spikes/s) with the cervical vagi intact or severed. Five and ten minutes after inhalation of 1% halothane, the cell activity (mean ± SE) expressed as per cent of the control was 55.3 ± 9 and 27 ± 7, respectively (P < 0.001), before bilateral cervical vagotomy. The corresponding values for 1.5% halothane were 25 ± 10.1 and 5.6 ± 5, respectively. Upon termination of halothane administration, the cell activity gradually returned toward the control level. The cell response to halothane was not affected by bilateral cervical vagotomy. Hypercapnia produced by inhalation of 5% CO₂ increased the cell activity, but halothane caused profound depression of the cells even in the presence of hypercapnia. Based on these results, it may be concluded that: 1) halothane has inhibitory effects on the activity of the inspiratory neurons of the NTS; and 2) halothane-induced respiratory depression has a central component and that the NTS may serve as a site of action of halothane for its respiratory depressant effect. (Key words: Anesthetics, volatile; anesthetic-induced respiratory depression; halothane; Brain: decerebration; inspiratory neuron; medulla oblongata; nucleus tractus solitarius. Ventilation: hypercapnia; hyperoxia.)

Respiratory depression is a common occurrence during administration of inhalation anesthetics such as halothane, enflurane, and isoflurane.1-3 The depressant action of halothane on respiration seems to be due to central as well as peripheral effects.4-6 The medullary respiratory neurons are an important part of the central respiratory organization because they process and integrate all the incoming information affecting respiration,7-9 and they serve as the site for the origin of efferent impulses to the respiratory muscles via spinal and cranial nerves.10-12 Any central respiratory depressant may conceivably influence the activity of these neurons. However, the direct effect of the inhalation anesthetics on these neurons has not been studied adequately.

Ngai et al.,4 while studying the respiratory effects of several anesthetics, showed that halothane elevated the electrical stimulus threshold of the medullary inspiratory center. Using single unit recording techniques, Kitahata et al.7 showed that administration of 0.5% and 1% halothane reduced the cell activity (spikes/s) of single inspiratory units of the solitarius complex by 43% and 58%, respectively. However, Kitahata et al. used cats already anesthetized with Dial® urethane, which could have masked to some extent the effect of halothane on the inspiratory neurons. Also, because the cats were breathing spontaneously, halothane-induced respiratory depression could have increased arterial carbon dioxide tension (PaCO₂), thus opposing the inhibitory effect of halothane on the respiratory neurons. To avoid these problems, we studied the effect of halothane on the activity of the inspiratory neurons of the nucleus tractus solitarius (NTS) of the medulla oblongata in decerebrate, paralyzed, mechanically ventilated cats before and after bilateral cervical vagotomy. Our results showed that 1% and 1.5% halothane produced significant depression of the activity of the inspiratory neurons before and after bilateral cervical vagotomy. Depression of cell activity also occurred in the presence of hypercapnia during halothane anesthesia.

Materials and Methods

Ten cats of either sex, weighing 2.5-3.5 kg, were anesthetized with halothane and nitrous oxide in oxygen. The trachea and femoral artery and vein were cannulated. The cervical vagi were isolated. The cats were then fixed in a stereotaxic instrument (David Kopf Instruments, Tujunga, California). Following decerebration according to the previously described method,13 halothane and nitrous oxide were turned off and the cats were paralyzed with intravenous gallamine triethiodide and ventilated with a Harvard ventilator using oxygen. Oxygen alone was used to minimize activity of the peripheral chemoreceptors. Approximately 3 h lapsed between termination of the halothane-nitrous oxide and the beginning of the study.

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HALOTHANE DEPRESSES INSPIRATORY NEURONS

Fig. 1. A (left). Reproduction from the oscilloscope tracing showing the phrenic neurogram (upper trace) and an inspiratory neuron discharge activity (lower trace). B (right). Polygraph recording of the phrenic neurogram, arterial blood pressure (ABP), tracheal pressure, and the integrated activity of an inspiratory neuron. The discharge activity of the unit in both A and B is in phase with that of the phrenic nerve; therefore, the unit is an inspiratory neuron.

Posterior fossa craniotomy was done to expose the medulla oblongata. The phrenic nerve was exposed and kept in a pool of paraffin to prevent drying. Bipolar silver wire electrodes were used to monitor the phrenic neurogram, which was recorded on a polygraph (Grass Instrument Company, Quincy, MA) and a cathode ray oscilloscope (CRO) (Tektronix, Beaverton, OR). A tungsten microelectrode (Frederick Haer and Co., Brunswick, ME) with a tip diameter of 1 μm and an impedance of 10 megohm, was inserted into the NTS with the aid of a hydraulic microdrive (David Kopf Instruments). The output of the microelectrode was amplified, passed through an amplitude discriminator (Quasitronics, Inc., Pittsburgh, PA), and monitored on the CRO (Tektronix). Any cell whose firing activity was synchronous with that of the phrenic nerve was considered an inspiratory neuron (fig. 1A). Further, the output from the amplitude discriminator was integrated and recorded on the Grass® polygraph as impulses (spikes) per s. The microelectrode signal was also fed to a Grass® audio monitor. Arterial blood pressure (ABP) and tracheal pressure were recorded on the Grass® polygraph. Thus, continuous recording of the firing frequency of the inspiratory neuron, phrenic neurogram, ABP, and tracheal pressure were made on the polygraph (fig. 1B) while the inspiratory neuron activity and phrenic neurogram were simultaneously displayed on the CRO as well. Whenever systolic ABP dropped below 100 mmHg during administration of halothane (four cats), an infusion of epinephrine was given to elevate ABP. Rectal temperature was monitored by a thermistor and kept between 37 and 38°C using a servocontrolled water mattress and heat lamps. End-tidal CO₂ was monitored by an infrared gas analyzer (Beckman Instruments, Schiller Park, IL) and maintained between 3.8 and 4.2%.

Halothane was administered using an anesthesia machine and a vaporizer (Fluotec III®, Cyprane, Keighley, Yorkshire). The oxygen flow was kept constant at 2 l/min throughout the experiment. The oxygen or halothane–oxygen mixture was introduced into the inspiratory port of the respirator. Before bilateral cervical vagotomy, all cats were given 1% halothane for 10–20 min. Five of the cats received 1.5% halothane as well for 10 min. Sufficient time was allowed between administration of 1 and 1.5% halothane for the inspiratory neuron activity to return to normal and remain so for at least 15 min. After bilateral cervical vagotomy, the same order of halothane administration was carried out. The Student's t test was used to analyze the data. When cell activity as per cent of control was compared with the control value, paired t test was used. When the activity of cells exposed to 1% halothane was compared with the activity of cells exposed to 1.5% halothane, unpaired t test was used. P values < 0.05 were considered statistically significant.

To ascertain the effect of hypercapnia on the response of the cells to halothane, two of the cats were given 5% carbon dioxide in oxygen for 5 min. At the end of the 5-min period, end-tidal CO₂ was measured and halothane–CO₂–O₂ mixture was administered for 10 min. This procedure was carried out before and after vagotomy.

Results

EFFECT OF HALOTHANE ON THE ELECTRICAL ACTIVITY OF THE INSPIRATORY NEURONS

One per cent halothane diminished the inspiratory neuron activity (spikes per s) before and after vagotomy (fig. 2). The degree of reduction was significant (P < 0.01)
that received CO₂–O₂ mixture. Inhalation of halothane
with the CO₂–O₂ mixture induced considerable depres-
sion of the cell activity, although its magnitude was less
relative to the cell depression produced by halothane–
oxxygen mixture (70% depression after 10 min exposure
to 1.5% halothane in O₂–CO₂ mixture vs. 88% depression
with 1.5% halothane in O₂). The recovery of the cell ac-
tivity was also faster in the presence of hypercapnia (80%
recovery 10 min after discontinuation of halothane from
halothane–O₂–CO₂ mixture vs. 50% recovery 10 min af-
after discontinuation of halothane from halothane–O₂ mix-
ture). It should be noted that in the presence of hyper-
capnia, there is increased cerebral blood flow, and there-
fore a faster time constant for wash-in and wash-out of
the anesthetic.

Discussion

The results show that halothane, in the concentrations
used, significantly decreases the activity of the inspiratory
neurons of the NTS. The inhibition was reversible, and
gradual recovery occurred when halothane was discon-
tinued. Further, the inhibition took place in the presence
of hypercapnia. Bilateral cervical vagotomy failed to pre-
vent the cell response to halothane inhalation.

CELL RESPONSE TO HALOTHANE IN THE
PRESENCE OF HYPERCAPNIA

Five minutes after the introduction of 5% CO₂ in ox-
ygen, the end-tidal CO₂ was 8% and 7% in the two cats

![Cell Activity (spikes/sec) as Percent of Control](image)

**Fig. 2.** Effect of administration of 1% halothane on the activity of
the inspiratory neurons of the nucleus tractus solitarius after bilateral
cervical vagotomy. Each point represents mean ± SE. The number of
cells studied at any given time is given in the parentheses. The amount
of cell depression becomes significant at 4 min (P < 0.01) and thereafter.
Cell recovery is displayed on the right-hand side starting at 5 min after
halothane discontinuation up to 20 min.

![Sequential polygraph tracings of the effect of 1.5% halothane](image)

**Fig. 3.** Sequential polygraph tracings of the effect of 1.5% halothane
on the activity of an inspiratory neuron after bilateral cervical vagotomy.
Gradual depression of the cell activity during halothane administration
is followed by gradual recovery as halothane is turned off. The ordinate
shows cell activity in spikes/s, and the abscissa shows time in min.
HALOTHANE DEPRESSES INSPIRATORY NEURONS

The inspiratory neurons were identified by the synchrony of their rhythmic discharge with that of the phrenic nerve, which is active only during inspiration. The inspiratory neurons, however, do not all fire simultaneously. In fact, according to their time of peak firing activity and duration of activity within the inspiratory phase of respiration, different types of inspiratory neurons have been recognized. Most of the neurons in the present study had their peak firing activity in early or mid-inspiration.

In the present experiments, we monitored the inspiratory concentrations of halothane. It would have been better to measure the end-tidal (alveolar) concentrations of halothane because it is the latter to which the arterial blood, and hence the brain, would equilibrate. Thus, in all likelihood, the greater depression of neural activity with 1.5% halothane compared with 1% halothane is due to the greater brain halothane partial pressure achieved with the former.

Reduction of the inspiratory neuronal activity by halothane may be translated into decreased efferent impulses to the inspiratory muscles leading to diminished tidal volume and hypventilation. The results suggest that NTS may serve as a site of action of the halothane-induced respiratory depression.

However, halothane may affect any of the respiration-related structures of the brain stem. These structures consist of pontine respiratory centers (pneumotaxic and apneustic); medullary respiratory neurons including dorsal respiratory group (DRG) associated with NTS; ventral respiratory group (VRG) associated with nucleus ambiguus (NA); nucleus para-ambiguus (NPA); nucleus retroambiguus (NRA); Bötzinger complex; and, finally, the chemosensitive area of the ventrolateral surface of the medulla. The respiratory neurons of the NTS are predominantly inspiratory and project into phrenic motoneurons that supply the diaphragm. The respiratory neurons in NA are mainly vagal motoneurons that innervate the laryngeal muscles, while those in NPA are mostly inspiratory whose axons synapse with phrenic and inspiratory intercostal motoneurons. The neurons of the NRA and Bötzinger complex are mostly excitatory and project to spinal motoneuron pools for expiratory intercostal and abdominal muscles.

Because the activity of the NTS neurons was significantly diminished by halothane, the question may arise as to how important the NTS neurons are in the overall act of breathing. The DRG located in the NTS was once proposed as the central pattern generator (CPG) for breathing. This hypothesis, however, has been since rejected, and the suggestion for an independent CPG for DRG neurons and an independent CPG for VRG neurons has been made. Furthermore, destruction of bilateral circumscribed regions of the medullary inspiratory complex by macrolesions, DRG and VRG neurons by microlesions, and NTS neurons without fibers of passage by kainic acid injection has failed to stop rhythmic breathing, although tracheal air flow, phrenic nerve activity, and minute ventilation, respiratory frequency, and sensitivity to CO₂ in anesthetized cats have been diminished. All these points to the fact that the CPG neurons and their location, which once was thought to be in the NTS, have not yet been identified, although presently they are postulated to be somewhere in the brain stem or spinal cord.

The fact that halothane decreased the inspiratory neuron activity in the presence of hypercapnia indicates that at least a portion of the diminished ventilatory response to CO₂ by halothane may be of central origin.

Our results are in general agreement with those of Kitahata et al., who showed that in Dial urethane-anesthetized cats, inhalation of 0.5% and 1% halothane reduced the cell activity by 43% and 58%, respectively.

There is evidence that halothane depresses the responses of the peripheral chemoreceptors to hypoxia and hypercapnia. It has also been shown that afferent fibers from the carotid body chemoreceptors project to the NTS inspiratory neurons. Therefore, it may be argued that the depressant action of halothane on the NTS neurons could be secondary to its inhibitory effect of the carotid body chemoreceptors. However, the following points contradict this argument. First, the cats in our experiment were ventilated with oxygen and therefore were hyperoxic. The carotid body chemoreceptor activity is minimal in hyperoxic conditions, and one would expect that its input to the NTS inspiratory neurons be minimal as well. Second, Berkenbosch et al. using the technique of artificial brain stem perfusion, demonstrated that the depressant effect of halothane on ventilation originates centrally as well as peripherally. Further, they attributed the peripheral effect to the action of halothane on the neuromechanical link between integrating centers and respiratory movements. Perhaps to better separate the peripheral effects of halothane from its central effects, these experiments should be carried out in peripheral chemoreceptor-denervated cats.

Our methodology may be employed to study the effects of inhalational anesthetics on other parts of the brain stem respiratory complex, such as VRG and its components. Thus, the differential sensitivity of the DRG and VRG neurons to anesthetics may be evaluated.

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References


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