Responses of Recurrent Laryngeal, Hypoglossal, and Phrenic Nerves to Increasing Depths of Anesthesia with Halothane or Enflurane in Vagotomized Cats

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In order to compare the responses to increasing depths of anesthesia with inhalational anesthetic agents of the phrenic nerve and cranial nerves supplying upper airway muscles, the effects of acute administration of halothane (2.5%) or enflurane (5.0%) on the activities of the hypoglossal nerve (HN), the recurrent laryngeal nerve (RLN), and the phrenic nerve (PN) were investigated in artificially ventilated, vagotomized cats. Following administration of halothane or enflurane, rhythmic inspiratory activities of all three nerves decreased progressively, but the decrease in HN activity was more pronounced than the decreases in HN and RLN activities. During the course of respiratory depression due to increasing depth of anesthesia with halothane or enflurane, rhythmic inspiratory activities of PN and RLN decreased linearly and approximately at the same rate, indicating that the response of RLN more closely resembles that of PN. However, at a relatively deep level of anesthesia, rhythmic inspiratory RLN activity was replaced by strong tonic RLN activity, while PN activity was completely abolished, causing quantitatively and qualitatively different responses of RLN and PN. These peculiar responses of RLN activity to increasing depth of anesthesia were examined in more detail with the use of single- or few-fiber preparations. Increasing depth of anesthesia depressed the inspiratory RLN units while recruiting other units, originally silent, indicating that there are two different groups of fibers in RLN whose response to increasing depth of anesthesia is exactly opposite to one another. The effects of halothane on HN, RLN, and PN were not identical to those of enflurane, since enflurane administration caused characteristic spike-like activities in HN and RLN, which were never observed following halothane, although the overall responses of the activities of all three nerves to increasing depth of anesthesia with halothane were similar to those with enflurane. The dissimilarities observed in all three nerves potentially could affect the balance of activity among upper airway and chest wall muscles and lead to obstruction in the upper airway. (Key words: Anesthetics, volatile: halothane; enflurane. Nerve: recurrent laryngeal; hypoglossal; phrenic.)

RECENTLY, the central control of the upper airway has received much attention. Obstruction of the pharyngeal or laryngeal airway during anesthesia results in alveolar hypventilation with profound ventilatory and cardiovascular sequelae. In a previous study, we demonstrated that the respiratory activity in the hypoglossal nerve (HN), which innervates the tongue muscles, is more vulnerable to the depressant effect of halothane than that in the phrenic nerve (PN), which innervates the diaphragm. Such differential suppression of HN and PN following an increase in the depth of anesthesia is in agreement with the commonplace clinical observation that deepening anesthesia causes the preferential relaxation of the tongue, leading to pharyngeal obstruction. The differential responses of these two nerves suggest that respiratory control of the tongue muscles and the diaphragm are mediated in part by differential neural pathways. The respiratory control of the larynx plays an auxiliary but important role in maintaining the upper airway. It is possible that the recurrent laryngeal nerve (RLN), a branch of the vagus that innervates the intrinsic laryngeal muscles, may be affected by anesthesia in a manner similar to HN, since both HN and RLN are cranial nerves. However, little is known about the sensitivity of RLN to anesthesia in comparison with PN and HN. The purpose of the present study is to compare the responses of HN, RLN, and PN with two commonly used inhalational anesthetic agents, halothane and enflurane.

Methods

Fourteen adult cats, weighing between 2.1 and 4.3 kg, were anesthetized with 2–3% halothane in O₂. The animals were fixed in a supine position, and their tracheas were cannulated with care to preserve the RLN on both sides. Catheters were placed in a femoral artery and vein. RLN, HN, and a root of PN were exposed in the right side of the neck, desheathed, and cut. Their central ends were placed on bipolar silver electrodes in a pool of warm liquid paraffin and prepared for recordings. The activities of all three nerves were amplified individually by a.c. amplifiers with 100-Hz and 3,000-Hz low- and high-frequency filters. The rectified signals of the amplified signals were integrated by a leaky R-C integrator, having a time constant of 100 ms.

In order to eliminate possible vagally mediated effects, bilateral vagotomy was performed by cutting the right vagus below the origin of the RLN and the left vagus in the mid-cervical region according to the technique described previously. In 10 of 14 cats, after surgical preparation, halothane was discontinued and replaced by α-chloralose (35 mg·kg⁻¹·iv). The animals then were paralyzed with pancuronium bromide (0.3 mg·kg⁻¹·iv) and
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artificially ventilated with 100% O₂ at a fixed rate and volume (rate × volume = 20 × 40 ml). The rectal temperature of the animals was maintained at about 38°C by a heating lamp. End-tidal P⁵CO₂ (PETCO₂) was monitored continuously with an infrared CO₂ analyzer (Minato® MEL RAS-41). RLN, HN and PN activities, their integrated activities, PETCO₂, and arterial blood pressure were all recorded on ultraviolet-sensitive paper.

The experiment was started at least 30 min after the discontinuation of halothane. PETCO₂ was maintained at 55–60 mmHg throughout the course of the experiment by introducing a small amount of CO₂ into the inspired gas to obtain vigorous inspiratory activity in all three nerves. When the animal was in a steady state condition, an arterial blood sample was taken and immediately analyzed for pH, P⁵CO₂, and P⁵O₂ with the use of an IL blood gas analysis system (IL system 1302).

Then, after obtaining 60 s of control data (baseline), the transient responses of HN, RLN, and PN activities to deepening anesthesia were examined by abrupt administration of either 2.5% halothane or 5.0% enflurane with the use of a precalibrated vaporizer (Fluotec Mark 2® for halothane and Muraco Enfluvick® for enflurane). Administration of either halothane or enflurane was continued until phasic PN activity disappeared. At this point, the inhalational agent was discontinued. The order of administration of halothane and enflurane was randomized. In order to minimize the residual effects of the previously administered agent, in each animal, sufficient time was allowed before the administration of the next agent to enable arterial blood pressure, RLN, HN, and PN activities to return to approximately the baseline level (90–100% of the baseline level). The intensity of RLN, HN, and PN activities was quantified by the peak height of the integrated activity. For the purpose of comparing various animals, percentage changes in nerve activities were calculated using a value of 100% for the activity during the control period. Because of the possibility that an interaction between α-chloralose (background anesthetic) and the test inhalational anesthetic agents might have caused the particular results, in another four cats baseline anesthesia was maintained with halothane (0.8% inspired concentration) or enflurane (1.6% inspired concentration) rather than with α-chloralose. In these animals the protocol was basically the same as the experiments with α-chloralose anesthesia. Thus, the transient responses of RLN, HN, and PN activities were examined by abruptly changing the inspired concentration of either halothane from 0.8 to 2.5% or enflurane from 1.6 to 5%, while PETCO₂ was maintained at 55–60 mmHg. Also, in seven cats (three cats anesthetized with α-chloralose and four cats anesthetized with 0.8% halothane), a single fiber or a few-fiber preparation was made from RLN by separating the whole nerve into fine filaments, and the effects of increasing depths of halothane anesthesia were tested. The conclusion that activities were from a single fiber was based on similar heights and wave forms of action potentials.

Statistical analysis was performed with the use of Student’s paired t test where appropriate.

Results

RESPONSES OF HN, RLN, AND PN ACTIVITIES TO HALOTHANE ADMINISTRATION

Average values of arterial pH, P⁵CO₂, and P⁵O₂ for 14 cats during the baseline state were 7.108 ± 0.059, 59.0 ± 3.0 mmHg, and 430 ± 35 mmHg, respectively. Figure 1 shows changes in PN, RLN, and HN activities following an acute administration and discontinuation of halothane in a cat anesthetized with α-chloralose. During the baseline state before administration of halothane, PN, RLN, and HN all showed vigorous activities in phase with inspiration. With the start of halothane administration, the activity of all three nerves, as well as arterial blood pressure, decreased progressively. During the course of respiratory depression due to increasing depth of anesthesia, the decrease in HN activity was more pronounced than the decreases in PN and RLN activity and rhythmic discharge of HN disappeared much earlier than those of PN and
RLN. In contrast with the rapid decrease in HN activity, the rate of decreases in PN and RLN activities was relatively slow. Both rhythmic PN and RLN activities decreased progressively up to the point in time where the rhythmic discharge of HN disappeared. After this time, the phasic inspiratory RLN activity was replaced gradually by an increasingly tonic RLN activity, while PN activity continued to decrease to the apneic point. The tonic RLN activity remained at a high level during a relatively deep level of anesthesia, whereas both PN and HN activities were virtually nil.

During the recovery period following discontinuation of halothane, with the resumption of phasic PN activity, the tonic RLN activity started to decrease and eventually was replaced by inspiratory phasic RLN activity. Thereafter, both PN and RLN activities progressively increased and returned to the baseline level within 25 min. Following administration of halothane, the PETCO₂ decreased somewhat, presumably because of a great depression of circulation. The change in PETCO₂ usually was small, although decreases in PETCO₂ by 2–4 mmHg were constantly observed in most of the animals.

The relationships of RLN and HN activities to PN activity following halothane administration for 10 animals anesthetized with α-chloralose are summarized in figure 2A. It can be seen that, compared with PN activity, HN activity is depressed preferentially in response to increasing depth of anesthesia, whereas the response of RLN is biphasic with an initial decrease followed by an increase in activity. The initial decrease of RLN in response to increasing depth of anesthesia is essentially proportional to the decrease of PN activity from the baseline state to approximately 20% of baseline activity.

**RESPONSES OF HN, RLN, AND PN ACTIVITIES TO ENFLURANE ADMINISTRATION**

The changes in the activities of all three nerves following administration and discontinuation of enflurane in a cat anesthetized with α-chloralose are shown in figure 3. Like the responses observed following halothane administration, the decline in HN activity was more pronounced than those in PN and RLN activities. PN and RLN responses were similar to each other until the phasic RLN activity was replaced by the tonic RLN activity. It also can be observed from figure 3 that rhythmic spike-like activities appeared transiently in the HN and RLN traces at a relatively deep level of anesthesia, although these activities usually were more dramatic in HN. These changes were consistently observed in all 10 animals.

The relationships of RLN and HN activities to PN activity following enflurane administration for all the animals anesthetized with α-chloralose are summarized in

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**Fig. 2.** The relationships of RLN and HN integrated activities to PN integrated activity following halothane administration (A) and enflurane (B) with background of α-chloralose anesthesia for 10 cats. Solid line (line of identity) indicates proportionality. Linear regression relationships of RLN and PN, calculated by the method of least squares, also are shown. *P < 0.01 (significantly different from the corresponding values of PN activity, paired t test). Values are mean ± SD.

**Fig. 3.** Changes in PN, RLN, and HN activities following administration of 5.0% enflurane. Administration and discontinuation of enflurane are indicated by arrows. A and B are continuous. For abbreviations see figure 1.
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FIG. 4. The relationships of RLN and HN integrated activities to PN integrated activity following a sudden change in inspired concentration of halothane (A) and enflurane (B) without background of α-chloralose anesthesia.

FIG. 5. Effects of increasing depth of anesthesia with halothane on the nerve activity recorded from PN and two different RLN filaments (I and II). The start of administration of 2.5% halothane began 1 min before the beginning of record A, and discontinuation of halothane is indicated by an arrow. A and B are continuous. Note that the tonic RLN discharge changes transiently into the rhythmic expiratory discharge before becoming silent during the recovery period (B).

EFFECTS OF DIFFERENT BACKGROUND ANESTHESIA

In order to examine the possible effects of different background anesthetics, halothane or enflurane was used as the background anesthesia in four cats. Changes in HN, RLN, and PN activities following an abrupt change in inspired concentration (halothane from 0.8 to 2.5% and enflurane from 1.6 to 5.0%) were qualitatively and quantitatively similar to those observed with a background of α-chloralose. Figure 4 shows the relationships of RLN and HN activities to PN activity obtained in these experiments. These results indicate that the use of α-chloralose as a background anesthetic does not mask the effects of increasing depth of anesthesia with halothane or enflurane.

EFFECTS OF INCREASING DEPTHS OF ANESTHESIA ON A SINGLE OR A “FEW-FIBER” PREPARATIONS OF RLN

In order to examine in more detail the biphasic responses of RLN activity to increasing depth of anesthesia, the effects of halothane on the activity of single- or few-fiber preparations of the RLN were tested in seven animals. Figure 5 illustrates the effects of halothane on two different RLN filaments, showing two different responses. During the course of respiratory depression due to halothane inhalation (fig. 5A), the inspiratory RLN units' activity as well as PN activity decreased progressively. After the disappearance of the inspiratory RLN units, a new unit, originally silent, started firing continuously. The reverse was true during the recovery period following discontinuation of halothane (fig. 5B).

Results were reproducible in different preparations. In total, 11 single-fiber (six inspiratory units and five originally silent units) and 12 few-fiber preparations containing both inspiratory and originally silent units were tested.
All the inspiratory units showed a progressive decrease in discharge and finally disappeared, while all the originally silent units showed a progressive increase in discharge in response to increasing depth of anesthesia with halothane.

Discussion

In the present study we used α-chloralose as a background anesthetic, since this agent is one of the most frequently used agents in animal experiments and is known to cause minimum depression of circulation and respiration. Although the interaction of the background anesthetic with other drugs is a problem inherent to experiments on anesthetized animals, the effects of deepening anesthesia with halothane or enflurane were quite consistent, whether the background anesthetics was α-chloralose, halothane, or enflurane. Therefore, it is unlikely that the use of α-chloralose masked the effects of increasing depth of anesthesia with inhalational agents.

A major finding in this study is that the responses of PN, HN, and RLN activities to increasing depths of anesthesia with inhalational anesthetic agents are all different. Results of the present study confirm our previous observation that increasing depth of anesthesia with halothane depresses HN activity more rapidly and to a greater extent than PN activity.1

Furthermore, the present study shows that increasing depths of anesthesia with enflurane also cause differential suppression of PN and HN activities. In contrast to the selective depression of HN activity to increasing depth of anesthesia, the responses of RLN in some ways more closely resemble those of PN. However, the finding that the behavior of RLN considerably diverges from that of PN at relatively deep levels of anesthesia indicates that there is also a qualitative difference in the responses of PN and RLN to increasing depth of anesthesia. The mechanism underlying this may be associated with the fact that RLN contains different groups of efferent fibers that innervate separately inspiratory (abductor) and expiratory (adductor) muscles of the larynx, while the fibers of PN innervate only an inspiratory muscle, the diaphragm. Thus, a possible explanation for the qualitative difference between RLN and PN responses is that increasing depths of anesthesia progressively depress inspiratory motoneurons while increasing the excitability of expiratory motoneurons. This explanation is not incompatible with our observations made in single-fiber or few-fiber preparations of RLN that increasing depths of anesthesia with halothane depressed the inspiratory RLN units while recruiting other units, originally inactive. Our observations also agree with the observation of Murtagh and Campbell9 that in goats the adductor muscles of the larynx show increased activity or spasm, while the abductor muscles are inactivated following administration of barbiturate, if the dose is great enough to cause apnea. Although the mechanisms may not be the same, similar responses of PN and RLN have been reported during hyperventilation. Eyzaguire and Taylor4 observed in anesthetized cats that hyperventilation blocked both vagal inspiratory and phrenic activity but increased vagal expiratory discharge. Bianconi and Raschi8 reported a similar observation in anesthetized cats showing increased activity of RLN fibers, which displayed an inspiratory discharge during spontaneous breathing, was inactivated completely by hyperventilation, while new fibers, originally inactive, were recruited. Also, Sherry and Megirian5 reported that in anesthetized or decerebrate cats, hyperventilation increased the tonic activity in the lateral cricoarytenoid muscle (adductor) while diminishing the inspiratory activity of the posterior cricoarytenoid muscle (abductor). In this connection there is evidence that removal of CO₂ constricts the larynx and leads to an increase in resistance to airflow.7

In addition to these observations concerning RLN-related activities, there is some evidence6,9 that the activity of HN declined more rapidly during removal of CO₂ than the activities of PN and RLN, whose responses are similar to each other. Thus, the qualitatively and quantitatively different responses of the activities of the three nerves observed in the present study are quite similar to the transient responses of these three nerves to CO₂ removal. In this connection it is important to note that the CO₂ response of the respiratory center is progressively depressed by increasing depths of anesthesia.10 The overall responses of PN, HN, and RLN to increasing depths of anesthesia with halothane and enflurane are similar. However, the finding that rhythmic spike-like activities are consistently observed after administration of a high concentration of enflurane regardless of whether the background anesthetic was α-chloralose or enflurane, whereas such activities were never observed following halothane administration, indicates that the effects of the two inhalational agents on the three nerves studied are not totally identical. The rhythmic spike-like activities following enflurane administration may be related to the well-known observation that enflurane produces characteristic electroencephalographic patterns and occasional motor movements interpreted as representing cerebral cortical irritability.11 It is interesting to note that the rhythmic spike-like activities usually started to appear when the inspiratory activity in HN was virtually lost and were more conspicuous in HN and RLN than in PN. These observations may suggest that the direct depression of hypoglossal motoneurons is not the principal cause of the selective depression of HN responses during enflurane
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administration. These observations also suggest that cranial nerves such as HN and RLN may receive more directly enflurane-induced cortical irritability than does PN.

Although it remains to be determined whether differences in the transient responses of PN, RLN, and HN similar to those observed in the present study can occur in humans, it is possible that upper airway obstruction during induction of anesthesia with inhalational anesthetic agents is attributable to the dissimilarities in the responses of these three nerves. Thus, the preferential depression of HN activity in response to increasing depth of anesthesia can relax the tongue muscles, leading to pharyngeal obstruction, whereas the activation of RLN activity at a relatively deep level of anesthesia can cause the constriction of the larynx, leading to laryngeal obstruction.

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References

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