Long-term facilitation of upper airway muscle activities in vagotomized and vagally intact cats

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Mateika, J. H., and R. F. Fregosi. Long-term facilitation of upper airway muscle activities in vagotomized and vagally intact cats. J. Appl. Physiol. 82(2): 419–425, 1997.—The primary purpose of the present investigation was to determine whether long-term facilitation (LTF) of upper airway muscle activities occurs in vagotomized and vagally intact cats. Tidal volume and diaphragm, genioglossus, and nasal dilator muscle activities were recorded before, during, and after one carotid sinus nerve was stimulated five times with 2-min trains of constant current. Sixty minutes after stimulation, nasal dilator and genioglossus muscle activities were significantly greater than control in the vagotomized cats but not in the vagally intact cats. Tidal volume recorded from the vagotomized and vagally intact cats was significantly greater than control during the poststimulation period. In contrast, diaphragm activities were not significantly elevated in the poststimulation period in either group of animals. We conclude that 1) LTF of genioglossus and nasal dilator muscle activities can be evoked in vagotomized cats; 2) vagal mechanisms inhibit LTF in upper airway muscles; and 3) LTF can be evoked in accessory inspiratory muscles because LTF of inspired tidal volume was greater than LTF of diaphragm activity.

vagal inhibitory memory; carotid sinus nerve; nasal dilator muscle; genioglossus muscle

LONG-LASTING ENHANCEMENT of ventilation, which is observed for up to 90 min after stimulation of the carotid bodies or carotid sinus nerve, is often referred to as long-term facilitation (LTF). LTF of ventilation and of phrenic and inspiratory intercostal nerve activities has been well documented in cats (6, 14, 15) and other animals (3, 8). However, few studies have examined LTF in upper airway motoneuron pools, and the results obtained have been variable. Jiang et al. (10) demonstrated that LTF of hypoglossal nerve activities is not present in decerebrate/decerebellate cats, whereas Bach and Mitchell (1) demonstrated LTF of hypoglossal nerve activity in intact rats. Although species differences might account for the variable results, it is possible that the absence of LTF in cat hypoglossal motoneurons was due to decerebellation of the animals, since Hayashi et al. (8) showed that LTF of phrenic nerve activity can be abolished in the rat after removal of the cerebellum. Therefore, LTF of upper airway motor activities may occur in the cerebellum-intact cat. Long-lasting facilitation of upper airway dilator muscles would be of functional significance because they play an important role in modulating upper airway flow resistance (2, 17, 18). Therefore, the first purpose of this investigation was to determine whether episodic carotid sinus nerve stimulation evokes LTF in the genioglossus and nasal dilator muscles of vagotomized, cerebellum-intact, spontaneously breathing cats.

The second purpose of this investigation was to evaluate the role of vagal afferents in the development and expression of LTF. To date, LTF of ventilation and of phrenic and hypoglossal nerve activities has been demonstrated primarily in vagotomized animals. It is conceivable that the degree of LTF observed in vagotomized animals might be reduced in intact animals by a long-lasting inhibitory memory, which could be elicited by repeated stimulation of lung stretch receptors, which would occur secondarily to the large tidal volume (Vt) changes that accompany each episode of carotid sinus nerve stimulation. This hypothesis is viable given that the activities of motor nerves innervating the nasal dilator, genioglossus, and diaphragm muscles are reduced in response to lung inflations (2, 9, 12) and increased when lung inflation is prevented in anesthetized vagally intact animals (16, 19). Furthermore, Xi et al. (21) reported that short-term potentiation of ventilation after exposure to hypoxia was significantly greater in dogs after their vagus nerves were cooled. Similarly, Gesell and Hamilton (7) showed that short-term facilitation of the phrenic nerve, which was elicited by stimulating the saphenous nerve, was obscured when the vagi were stimulated simultaneously. Despite this supporting evidence, no investigation has been designed to determine whether a long-lasting inhibitory vagal memory is evoked as a consequence of the increased Vt that accompany repeated episodes of carotid sinus nerve stimulation. Therefore, the second purpose of this investigation was to determine whether LTF, induced by carotid sinus nerve stimulation, is attenuated in animals with intact vagus nerves.

METHODS

Animal preparation. Acute experiments were performed on 16 supine cats of either sex. The mass of the animals ranged from 2.35 to 4.5 kg. The animals were anesthetized initially with a mixture of 2–3% halothane in oxygen, and the femoral arteries and veins were cannulated for blood pressure monitoring, withdrawal of arterial blood samples, and the intravenous infusion of bicarbonate, anesthetic agents, and fluids. Subsequently, halothane anesthesia was gradually discontinued while a bolus of α-chloralose (35–50 mg/kg) and urethane (200 mg/kg) was administered intravenously. We ensured that an effective analgesic level was maintained throughout the transition by monitoring the withdrawal and pupillary reflexes of the animal.

After surgical anesthesia was achieved, the trachea was cannulated for the measurement of airflow, and a midline laparotomy was performed to expose the diaphragm. Staining...
less steel bipolar recording electrodes (California Fine Wire) were inserted into the costal diaphragm, the nasal dilator, and genioglossus muscles. The genioglossus muscle was accessed via the floor of the mouth. Eight of the cats were then vagotomized bilaterally at the midcervical level, whereas the remaining cats were studied with vagus nerves intact. In all experiments, a carotid sinus nerve was isolated and prepared for stimulation by placing it across bipolar platinum electrodes, which were positioned as far from the glossopharyngeal nerve as possible.

Measurement of respiratory, cardiovascular, and electromyographic (EMG) parameters. Bidirectional airflow was measured with a pneumotachometer (model 8411, Hans Rudolph) that was attached to the tracheal cannula. To deliver the desired mixture of O$_2$, CO$_2$, and N$_2$ to the animals during the experimental protocol, the outflow port of a rotameter was attached to the pneumotachometer with a T tube system, which has been described previously (5). The fractional concentrations of end-tidal CO$_2$ and O$_2$ were sampled at the tracheal cannula by using rapidly responding CO$_2$ (model CD-3A, Ametek) and O$_2$ (model OM-11, Bedackman) analyzers. Rectal temperature was monitored and maintained at 37.5 ± 0.5°C by using a servo-controlled heating lamp (model 73A, Yellow Springs Instruments). Blood pressure was measured with a saline-filled catheter transducer (P23XL, Gould) that was attached to a femoral arterial cannula.

Arterial blood samples (0.3–0.5 ml/sample) were withdrawn and analyzed immediately for arterial P$_{O_2}$ (P$_{O_2}$) and P$_{CO_2}$ (P$_{CO_2}$) and for pH (model BGM, Cameron Instrument). The blood gas and pH values were corrected by using the rectal temperature that was recorded at the time of sampling. Bicarbonate values were calculated by using measured pH and P$_{CO_2}$ values, and if a base deficit existed it was corrected by infusion of sodium bicarbonate intravenously.

The EMG signals recorded from the diaphragm, nasal dilator, and genioglossus muscles were amplified and filtered (30–3,000 Hz) with alternate current-coupled differential amplifiers (model 7P511, Grass Instruments). The analog signals were converted to digital signals (model 4000, Vetter Digital) and stored on videocassette tape. In addition, the integrated EMG (iEMG) activities (Coulbourn S76–01, time constant 100 ms), stimulus output, airflow, integrated airflow (model 7P10, Grass Instruments), arterial pressure, and end-tidal CO$_2$ and O$_2$ were monitored and recorded continuously on a polygraph (model RP57C, Grass Instruments).

Experimental protocol. After completion of the surgery, the preparation was given at least 30 min to stabilize. During this interval, the animal inspired pure O$_2$ (inspired O$_2$ fraction = 1.0). After the stabilization period, control conditions for the diaphragm, nasal dilator, and genioglossus muscles were established by setting the end-tidal CO$_2$ level ~2 Torr above the threshold for respiratory-related upper airway muscle activity. We attempted to maintain this level throughout the protocol by altering the flow of CO$_2$ originating from the rotameter. Hence, fluctuations in end-tidal CO$_2$ were minimized despite changes in body temperature, blood pressure, and metabolic rate. Control data were then recorded for at least 4 min. The isolated carotid sinus nerve was then stimulated five times with 2-min trains of constant current (25 Hz, 0.5-ms duration). The current was set at three times the threshold current for carotid sinus nerve stimulation-induced upper airway and chest wall muscle activation. Each train was separated by a 5-min recovery period. Before, during, and for 60 min after the fifth stimulation episode, the physiological variables mentioned above were recorded continuously. After completion of the experimental protocol, the end-tidal level of CO$_2$ was gradually increased in an attempt to measure maximal Vt and EMG activities of the nasal dilator, genioglossus, and diaphragm muscles. We assumed that maximal EMG activities were achieved if further increases in end-tidal CO$_2$ were not accompanied by further changes in EMG activity. Furthermore, the carotid sinus nerve was stimulated during hypercapnia to unequivocally ensure that maximal activities were obtained. Arterial blood was sampled before each stimulation period and at 1 min, 5 min, and every 10 min after the fifth stimulation period.

Data analysis. Ten breaths recorded immediately before the first stimulation period and at intervals corresponding to 7.5, 15, 30, and 60 min after the fifth stimulation period were used to measure inspiratory duration (Ti), expiratory duration (Te), and inspired Vt. Breathing frequency was calculated from measurements of Ti and Te. Mean arterial blood pressure (MAP) and peak iEMG activities recorded from each muscle were measured for the corresponding 10 breaths. The iEMG activities and Vt were expressed as a percentage of maximal activity. In addition, Vt and the iEMG activities recorded at the selected poststimulation time intervals were subtracted from the average value of the 10 breaths recorded during the control period and reported as the difference from control (see Fig. 4). Mean values for each physiological variable were then calculated for each 10-breath epoch. Subsequently, a group mean value was calculated for the vagotomized and vagally intact animals.

A one-way analysis of variance with repeated measures was used to determine whether a significant difference existed between each time interval (control, 7.5, 15, 30, and 60 min, respectively) within a given group (vagotomized and vagally intact). If the absolute values obtained for a physiological variable were not normally distributed, Friedman’s repeated measures analysis of variance was employed. If a significant difference existed among time intervals, individual comparisons were made by using the Student-Newman-Keuls post hoc test. Unpaired t-tests or their nonparametric equivalent, the Mann-Whitney U-test, were used to determine whether differences existed between intact and vagotomized cats at selected time intervals. The Bonferroni correction factor for multiple comparisons was applied. All values in the text and figures are presented as means ± S.E., and the level of significance chosen was P < 0.05.

RESULTS

Figures 1 and 2 show Vt and the iEMG activities recorded from a vagotomized and vagally intact cat, respectively, before, during, and after carotid sinus nerve stimulation. Note that LTF of nasal dilator and genioglossus muscle activities occurs throughout the poststimulation period in the vagotomized cat (Fig. 1) and that this response is absent during the poststimulation period in the vagally intact cat (Fig. 2).

Figure 3 shows the average inspired Vt, and diaphragm, genioglossus, and nasal dilator muscle iEMG activities that were recorded from the vagotomized and vagally intact cats under control conditions. Control conditions were established by setting the end-tidal CO$_2$ level ~2 Torr above the threshold for respiratory-related upper airway muscle activities. This figure demonstrates that the Vt and diaphragm activities recorded from both groups were significantly greater than the upper airway muscle activities when all the values were expressed as a percentage of the maximal response to hypercapnia. This occurred because the
CO₂ level required to activate the upper airway muscles was significantly greater than the eupneic CO₂ level in these spontaneously breathing cats. Figure 3 also demonstrates that the VT measured from the vagotomized animals was greater than the values measured from the vagally intact animals. The maximum VT that was measured in response to hypercapnia at the end of each experiment averaged 127.4 ± 22.6 ml in the vagotomized cats and 95.9 ± 14.5 ml in the vagally intact cats, respectively.

Figure 4 shows the average values for VT and diaphragm, nasal dilator, and genioglossus muscle iEMG activities recorded from the vagotomized and vagally intact cats during the poststimulation period. The VT and iEMG activities were standardized by calculating the difference between the poststimulation values and
the control values shown in Fig. 3. Figure 4 demonstrates that the average VT and nasal dilator and genioglossus muscle iEMG activities were significantly greater throughout the poststimulation period in the vagotomized cats, whereas diaphragm activities did not change significantly.

Although similar diaphragm and VT responses were observed in the vagally intact cats (Fig. 4), no significant increases in nasal dilator and genioglossus muscle iEMG activities were recorded during the poststimulation period. Consequently, nasal dilator and genioglossus muscle activities recorded during the earlier segments of the poststimulation period were significantly less than the values calculated for the vagotomized animals. The magnitude of this difference diminished during the latter half of the poststimulation period as indicated by the gradual increase in iEMG activities that were recorded from the vagally intact cats. Figure 5 shows that the changes in VT and muscle activities recorded from the vagotomized and vagally intact animals were not accompanied by significant changes in Ti, Te, or breathing frequency.

Figure 6 shows that fluctuations in PaCO2 were minimized throughout the experimental protocol and poststimulation period. PaCO2 values recorded from the vagally intact animals tended to be greater than the values recorded from the vagotomized animals, although this difference was not significant. PaO2 was maintained at hyperoxic levels in all experiments (range 217–472 Torr). No significant change in MAP occurred during the initial 30 min of the poststimulation period. However, the decrease in MAP at 60 min poststimula-

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**Fig. 3.** Histograms showing average VT (filled bars) and diaphragm (open bars), nasal dilator (hatched bars), and genioglossus muscle (cross-hatched bars) iEMG activities recorded from vagotomized and vagally intact cats during prestimulus control conditions. Values are means ± SE (n = 8 vagotomized cats and 8 vagally intact cats). VT and iEMG activities were expressed as % maximal activity (see Figs. 1 and 2). Significantly different from nasal dilator and genioglossus muscle activities, *P < 0.05. Significantly different from VT values calculated for vagally intact cats, ‡P < 0.05.

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**Fig. 4.** Line plots showing average VT (C) and diaphragm (A), nasal dilator (B), and genioglossus muscle (D) iEMG activities recorded from vagotomized (closed symbols) and vagally intact cats (open symbols) at various time intervals after 5th stimulation period. Values are means ± SE (n = 8 vagotomized cats and 8 vagally intact cats) expressed as % maximal activity and then standardized by calculating difference between each time point and recorded prestimulus control value. Significantly different from control, *P < 0.05. Significantly different from vagotomized groups, ‡P < 0.05.
tion reached statistical significance in the vagotomized cats. There was no significant difference in the MAP values recorded from the vagally intact and vagotomized animals.

**DISCUSSION**

The present investigation showed that LTF of genioglossus and nasal dilator muscle activities can be evoked by carotid sinus nerve stimulation in cerebellum-intact vagotomized cats and that LTF is inhibited in vagally intact cats. The results also show that LTF of inspired Vt exceeds that of diaphragm EMG activity, although this latter finding may only pertain to situations when PaCO₂ is relatively high.

Critique of methods. The facilitation of Vt and EMG activities recorded during the poststimulation period might have been elicited by at least three mechanisms other than the LTF mechanism. First, a decrease in the depth of anesthesia may have evoked the observed responses. However, if this occurred, the activities recorded from the vagotomized animals would have gradually increased throughout the poststimulation period. In contrast, the responses recorded remained constant or decreased throughout this interval. Furthermore, if changes in the depth of anesthesia were responsible for the observed results, similar responses would have been observed in the vagotomized and vagally intact cats. Second, a gradual increase in PaCO₂ may have caused the long-lasting facilitatory response recorded during the poststimulation period. However, the changes in PaCO₂ were minimized and often did not vary by more than ±1 Torr compared with baseline levels, in both the vagotomized and vagally intact cats. Third, a decrease in MAP could have increased the PCO₂ of the extracellular fluid that surrounds the central chemoreceptors despite the maintenance of PaCO₂. This probably did not occur in this investigation because MAP was stable in the vagally intact group throughout the poststimulation period. Furthermore, MAP remained stable in the vagotomized group for 30 min poststimulation, and although MAP decreased significantly at 60 min poststimulation it was not accompanied by any significant change in Vt or EMG.
activities. Given the above arguments, we believe that LTF was responsible for the changes in VT and upper airway muscle activities that were observed after episodic stimulation of a carotid sinus nerve.

Nevertheless, it has been suggested that electrical stimulation of carotid sinus nerves may introduce a nonphysiological stimulus to the central nervous system because the fibers that form this nerve rarely fire synchronously at the frequency (25 Hz) employed in this investigation (13). However, previous investigations have shown that repeated episodes of isocapnic hypoxia (which is a more physiologically relevant stimulus than carotid sinus nerve stimulation) can also elicit LTF of phrenic and hypoglossal nerve activities in vagotomized animals (1, 8, 14). Thus our results, which were obtained with electrical stimulation of a carotid sinus nerve, are consistent with data obtained by subjecting animals to isocapnic hypoxia.

Carotid sinus nerve stimulation-induced LTF of upper airway and inspiratory muscles in vagotomized cats. A number of investigations have shown that LTF of phrenic and inspiratory intercostal nerve activities can be elicited in vagotomized animals by stimulation of the carotid bodies or carotid sinus nerve (1, 3, 8, 14). In contrast, whether or not episodic carotid sinus nerve stimulation induces LTF in upper airway motoneurons is uncertain. Jiang et al. (10) found no evidence for carotid sinus nerve stimulation-induced LTF of hypoglossal nerve activity in decerebrate/decerebellated cats, whereas Bach and Mitchell (1) observed hypoxia-induced LTF of hypoglossal nerve activity in intact rats. Bach and Mitchell (1) suggested that this difference was not species dependent but was caused by decerebellation of the cats used in the Jiang et al. (10) study. This hypothesis is supported by the results obtained from the present investigation, which showed that LTF of genioglossus and nasal dilator muscle activities can be evoked in the cerebellum-intact, vagotomized, spontaneously breathing cat. Genioglossus and nasal dilator muscle activities increased by 15% compared with baseline values. This increase is similar to values reported for the LTF recorded from inspiratory intercostal nerves, which increased on average 20% above baseline values after episodic carotid sinus nerve stimulation (6). Thus the cerebellum may have a role in the development of LTF in upper airway muscles. The importance of the cerebellum in the development of LTF was demonstrated previously by Hayashi et al. (8), who showed that carotid sinus nerve stimulation-induced LTF cannot be elicited in the phrenic nerve of decerebellated rats.

Although LTF was observed in the iEMG activities of the upper airway muscles, no significant facilitation of mean diaphragm muscle activity was recorded in the vagotomized cats. It is possible that the diaphragm muscle activities, which were elevated under control conditions because we increased $P_aCO_2$ levels to elicit respiratory-related upper airway muscle activities (see Fig. 3), may have obscured LTF due to saturation of phrenic motoneuronal activity. This suggestion is supported by the findings of Eldridge et al. (4), who showed that phrenic nerve activities can saturate as respiratory drive is increased with hypercapnia.

Nevertheless, this explanation does not account for the varying degree of LTF of VT and diaphragm activities because the activities of these two variables were similar under control conditions. The significant LTF of inspired VT, which presumably was generated by the activation of primary and accessory inspiratory muscles, may have occurred because the effect of serotonergic inputs on accessory muscle motoneurons might be greater than the effect on phrenic motoneurons. This suggestion is supported by the findings of Fregosi and Mitchell (6), who showed that LTF of inspiratory intercostal nerve activities was greater than that recorded from the phrenic nerve and that LTF recorded from these two nerves was mediated by a serotonergic mechanism. Similarly, Jiang and Shen (11) found a greater density of serotonergic terminals on inspiratory intercostal motoneurons compared with that on phrenic motoneurons.

Carotid sinus nerve stimulation-induced LTF of upper airway and inspiratory muscles in vagally intact cats. We hypothesized that the degree of LTF of inspiratory and upper airway muscle activities observed in vagotomized animals might be reduced in intact animals by a long-lasting inhibitory memory, which could be elicited by repeated stimulation of lung-stretch receptors. This hypothesis was based on the observation that the activities of motor nerves innervating the nasal dilator, genioglossus, and diaphragm muscles are reduced in response to lung inflations (2, 9, 12) and increased when lung inflation is prevented (16, 19). Furthermore, Xi et al. (21) reported that short-term potentiation of ventilation after exposure to hypoxia was significantly greater in dogs after the vagus nerves were cooled. Finally, Bach and Mitchell (1) and Hayashi et al. (8) showed that episodic hypoxic stimulation of the carotid bodies evoked simultaneously a long-term inhibitory memory and LTF of phrenic and hypoglossal nerve activities. In their studies, LTF was attenuated during the early part of the posthypoxic period before gradually increasing as the inhibitory memory diminished. The present investigation also suggests that a long-lasting vagal inhibitory memory can be activated by repeated stimulation of lung-stretch receptors because LTF of upper airway muscle activities in the vagally intact cats was significantly less compared with the responses recorded in the vagotomized cats during the initial portion of the poststimulation period.

However, it is of interest that the inhibitory memory did not attenuate VT or diaphragm activities. There are at least two explanations for the observed differences between upper airway and chest wall muscle activities. First, many investigators have suggested that afferent influences from stretch receptors are differentially distributed to motoneurons innervating upper airway muscles and the diaphragm (9, 16, 19). This hypothesis is based on the observation that periodically withholding lung inflation in decerebrate servo-ventilated cats results in a greater augmentation of hypoglossal and facial nerve activities compared with that recorded...
from the phrenic nerve (9). Second, the effect of the vagal inhibitory memory on diaphragm and especially accessory inspiratory muscle motoneurons may have been overridden by the elevated level of excitation of these motoneuron pools (6). This suggestion is supported primarily by the Vt response that was observed in the vagally intact cats during the poststimulation period. Vt was significantly greater than control throughout the poststimulation period, in contrast to the response measured from the upper airway muscles. Nevertheless, the pattern of development was similar to that observed for the upper airway muscles, in that LTF of Vt gradually increased throughout the poststimulation period. This finding suggests that the inhibitory memory may have been present during the initial portion of the poststimulation period but was overridden by excitatory inputs to the accessory muscle motoneurons.

Physiological significance. The physiological significance of LTF of upper airway muscle activities in humans is uncertain. However, Bach and Mitchell (1) suggested recently that this mechanism may be activated in individuals with obstructive sleep apnea. This syndrome is characterized by periodic narrowing of the upper airway during sleep, which may be accompanied by arterial hypoxemia. Thus repeated episodes of hypoxemia could activate the upper airway motoneuron pools via the carotid bodies, the result being an increase in upper airway muscle tone that could contribute to maintenance of upper airway patency. If this hypothesis is correct, then upper airway muscle iEMG activities during sleep should be greater in individuals with obstructive sleep apnea. Support for this hypothesis comes from the findings of Suratt et al. (18), which showed that alae nasi and genioglossus muscle activities during sleep are greater in patients with obstructive sleep apnea than in control subjects.

Given the findings of the present investigation, a long-lasting inhibitory vagal memory could also be activated in individuals with obstructive sleep apnea because the termination of most apneic events is accompanied by marked elevations in Vt. Repeated elevations of Vt could conceivably activate the vagal inhibitory memory. Activating this memory could cause further instability of the upper airway because of a reduction in muscle tone, if this mechanism was more dominant than LTF. However, the vagal inhibitory memory may be less dominant than the LTF mechanism in humans because the relative strength of afferent stretch-receptor input may be less in humans than in other mammals (20).

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