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INVITED LECTURES

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CHEMORECEPTOR AND VAGAL INFLUENCES ON GENIOGLOSSAL MUSCLE RESPONSES TO INSPIRATORY RESISTIVE LOAD

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The present study was undertaken to determine the mechanisms underlying the involvement of upper airway dilating muscles in compensatory responses to added inspiratory resistive load. Experiments were performed in tracheostomized, anaesthetised rabbits. The effect of inspiratory resistive loading on the electromyographic activity of the genioglossus muscle, the major dilating muscle of the pharynx, was studied in vagotomized and vagally intact rabbits during spontaneous breathing with a hypoxic gas mixture (10% O_2 in N_2) or oxygen. In the vagally intact animals the peak value and duration of genioglossus muscle inspiratory activity increased in the first loaded breath before any noticeable change in the arterial blood gases. Hyperoxia decreased, whereas hypoxia increased the immediate response of the genioglossus activity to inspiratory loading. Removal of vagal volume-related feedback (by vagotomy) significantly increased the genioglossus muscle activity; the increase being more under hypoxia than under hyperoxia. In contrast to vagally intact animals, there was no first-breath increase in genioglossus activity during loading. The results indicate that the immediate involvement of the genioglossus muscle in response to inspiratory resistive load is mediated by vagal-volume feedback. Baseline oxygen tension before loading modulates the immediate reflex vagal-related response of the genioglossus muscle.

Key words: *genioglossus muscle, inspiratory resistive load, vagotomy, upper airways*

INTRODUCTION

Chronicle obstructive pulmonary diseases (COPD) are associated with an increase in the mechanical load. However, although the work of breathing is known to increase in COPD, ventilation is preserved and PCO_2 is usually normal until the very hard stages of the disease, because neuromuscular load compensation takes place (1, 2).

Load compensation is effected through an increase in inspiratory activity of pressure-generating muscles (e.g., diaphragm, intercostals), which is followed by increased negative pressure in upper airways and may induce upper airway obstruction. The segment of the upper airway most vulnerable to obstruction is the pharynx. Adequate ventilation of the lung depends on the coordinated activity of pressure-generating muscles and pharynx airway dilating muscles, which maintains patency of airways and allows "pumping muscles" to draw air into lungs (3, 4, 5). However, very little is known about the participation of these muscles in compensation for added mechanical load.

Our recent data have indicate that inspiratory activation of the genioglossus muscle, the major pharynx dilating muscle, together with others pharyngeal muscles is required for successful compensation of the added inspiratory load. The recruitment of these muscles decreases airway resistance during loaded breathing and thereby facilitates load compensatory function of "pump" muscles (6). The present study was designed to determine the mechanisms of the genioglossus muscle recruitment induced by inspiratory resistive loading. Laboratory investigations have shown that inspiratory activation of the genioglossus muscle is influenced by upper airway mechanoreceptor activation from negative pressure in the pharynx (7, 8, 9, 10), chemoreceptor stimulation from hypoxia and hyperoxia (11, 12, 13), and by the vagal volume-related feedback arising from lung inflation (5, 14, 15, 16). However, the relative contribution of each of the above mechanisms to the recruitment of pharynx dilating muscles by mechanical load is unclear.

Our previous study has demonstrated that activation of upper airway receptors by negative pressure is not required for the involvement of the genioglossus muscle in the load response (6). Additionally, it has been shown that in tracheostomized subjects, whose upper airways were functionally separated, ventilatory responses to inspiratory mechanical loads are preserved in the absence of afferent information from the upper airways (17). These data allow supposing, by analogy to the diaphragm, that the major mechanisms of the involvement of pharynx dilating muscles in the compensatory load response are weakness of the inhibition from a vague-volume feedback (smaller tidal volume during load breathing) and an increase in chemoreceptor drives. To examine this hypothesis we investigated the effects of vagal volume-related feedback on the genioglossus muscle involvement in the load compensatory responses under normoxia, hypoxia, and hyperoxia.

MATERIAL AND METHODS

Experiments

All animal procedures were conducted in accordance with the ethical guidelines of the European Community Council Directives 86/609/EEC. The experiments were performed on 18 tracheostomized rabbits (average weight 2.5 ±0.4 kg), which were anesthetized with urethane (1000

mg kg^{-1}, ip). The level of anesthesia was sufficient to eliminate pain reflexes. Assessing corneal reflex and responses to tactile stimuli monitored the depth of anaesthesia. Tracheostomy was performed through a midline neck incision. A tracheal cannula was inserted into a distal part of trachea bellow the larynx.

In the first series of experiments, the role of vagal volume-related feedback in the load response was studied. An inspiratory resistive load was applied to each animal before and after vagotomy during breathing with room air (normoxia). The animals were vagotomized bilaterally at the midcervical level. The load response was defined as an increment in peak electromyographic (EMG) activity in response to loading.

In the second series of experiments, comparisons were made between the load responses obtained during spontaneous breathing with a hypoxic gas mixture (10% O_2 in N_2) and with oxygen. For the same experiment, each animal breathed with pure oxygen followed by room air, and then by hypoxia. The inspiratory resistive loading was performed after 10 min breathing with oxygen or hypoxic mixture.

Loads and esophageal pressure

Clamping of the inspiratory tube and using of valves, which allowed expiration without added resistive load, induced only inspiratory loading. The inspiratory load was measured as the oesophageal pressure (P_{ES}) in the first loaded inspiration and was expressed as the percentage change of the unloaded P_{ES}. A moderate inspiratory load (110% P_{ES}) was applied for 10 min in the experiments of the first series of. In the second series, maximal resistive load was applied (5 occlusion efforts).

A latex balloon positioned in the lower one-third of the oesophagus allowed measuring oesophageal pressure swings. The balloon was filled with 0.5 ml of air and connected via catheters (inner diameter of 1.5 mm, length 50 cm) to a differential pressure transducer.

Muscle activity measurements

EMG of the genioglossus and diaphragm muscles were obtained from bipolar fine wire electrodes. The electrodes, inserted into the genioglossus muscle, were placed halfway between the chin and the hyoid bone. Laparotomy was performed for the implantation of electrodes into the right costal part of the diaphragm. Then the laparotomy incision was closed in layers. The electrical activity of both muscles was filtered at band-pass frequencies of 10-1000 Hz, amplified, and integrated by means of a resistance-capacitance (R-C) circuit whose time constant was 0.1 s.

Ventilation and alveolar gases

Airflow was measured by a pneumotachograph head (Fleisch no. 0) and integrated to volume. The value for minute ventilation was calculated from the mean tidal volume and respiratory frequency of ten respiratory cycles. The partial pressures of CO_2 ($P_{ET}CO_2$) and O_2 ($P_{ET}O_2$) in the alveolar gas were measured from the peak end-tidal values recorded by a mass spectrometer (MX 6202). In the control condition during breathing room air, $P_{ET}CO_2$ was 35 ±1 mmHg and $P_{ET}O_2$ was 85 ±3 mmHg. For 10 min breathing with oxygen, $P_{ET}O_2$ rose above 200 mmHg, and $P_{ET}CO_2$ increased to 40 ±2 mmHg. For 10 min breathing with the hypoxic gas mixture, $P_{ET}O_2$ fell to 48 ±5 mmHg and $P_{ET}CO_2$ decreased to 30 ±4 mmHg.

Data analysis

Data were expressed as the percentage change from the control value (mean ±SE). Control measurements were performed during unloaded spontaneous breathing. Statistical analysis was performed by using the non-parametric Wilcoxon signed-rank test. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Respiratory response to inspiratory resistive load in vagally intact and vagotomized animals.

Table 1 demonstrates indices of the breathing pattern in the anesthetized, vagally intact and vagotomized, animals during unloaded tracheostomy breathing. The inspiratory resistive load evoked a significant decrease in maximal inspiratory flow (-84%) and a reduction in minute ventilation (-70%). The latter resulted from lower both tidal volume (-60%) and respiratory rate. The decrease in respiratory rate resulted from an increase in inspiratory time (+34%). Expiratory time did not change significantly. With time, tidal volume and ventilation reverted back toward the baseline values, although the return was not complete.

Table 1. Indices of breathing pattern in vagally intact and vagotomized rabbits during unloaded breathing via tracheostomy.

	V_T (ml)	f (min^{-1})	V_E (ml min^{-1})	P_{ES} (mm H_2O)	E_{DI} (%)	E_{GG} (%)
Vagally intact	19 ±2.4	59 ±5.5	1121 ±135	-10 ±2	100	100
Vagotomized	25 ±2.6*	41 ±3.4*	1025 ± 82	-15 ±2	131 ±12*	316 ±35*

V_T - tidal volume; f - inspiratory rate; V_E - minute ventilation; P_{ES} - peak esophageal pressure; E_{DI} and E_{GG} - peak value of integrated activity of the diaphragm and genioglossus, respectively. Values are means ±SE. *$P<0.05$ between vagally intact and vagotomized animals.

During resting unloaded breathing, the genioglossus muscle exhibited faint inspiratory discharges that began simultaneously with diaphragm discharges. In vagally intact rabbits, both genioglossus and diaphragm EMG activities increased in magnitude and duration in the first loaded inspiration (*Fig. 1*). The peak genioglossus EMG activity and the duration of its inspiratory discharges increased to 124 ±13% and 113 ±5% of control, respectively ($P<0.05$). The peak value of diaphragm EMG activity increased to 106 ±1% of control in the first loaded effort ($P<0.05$). The difference between the genioglossus and diaphragm EMG responses to loading was statistically significant ($P<0.01$).

The immediate muscle response to loading, in the first loaded breath, is not related to any change in the arterial blood gases, because the chemoreceptor drive increases only during the multibreath loading. Thus, the immediate genioglossus recruitment induced by the loading is a neural reflex response. As the experiments were performed in tracheostomized animals, the contribution of the upper airway receptors to the activity of the genioglossus muscle may also be excluded. Moreover, other studies have demonstrated that upper airway receptors do not contribute to the ventilatory and EMG responses to resistive load (6, 17).

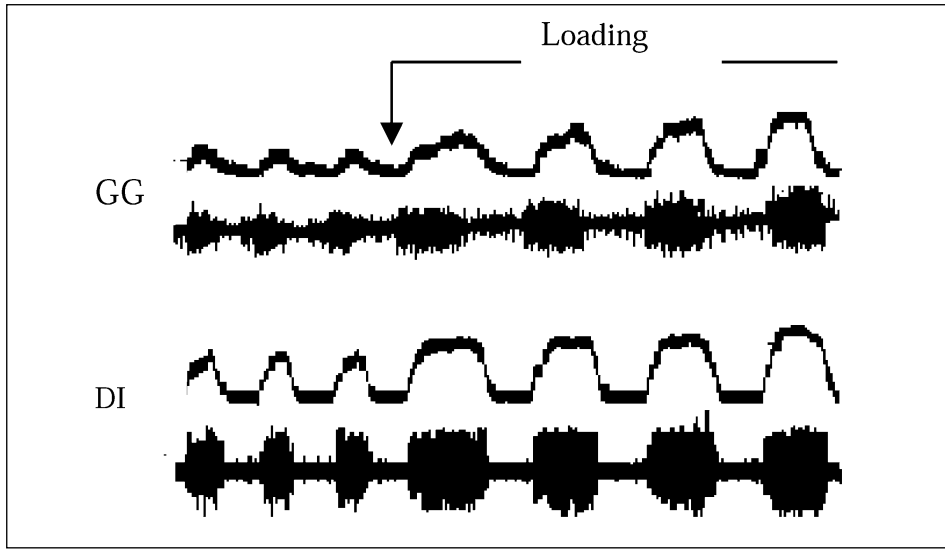

Fig.1. EMG response to inspiratory resistive loading in a vagally intact rabbit. Representative recordings of native and integrated electrical activity from the genioglossus (GG) and diaphragm (DI) muscles.

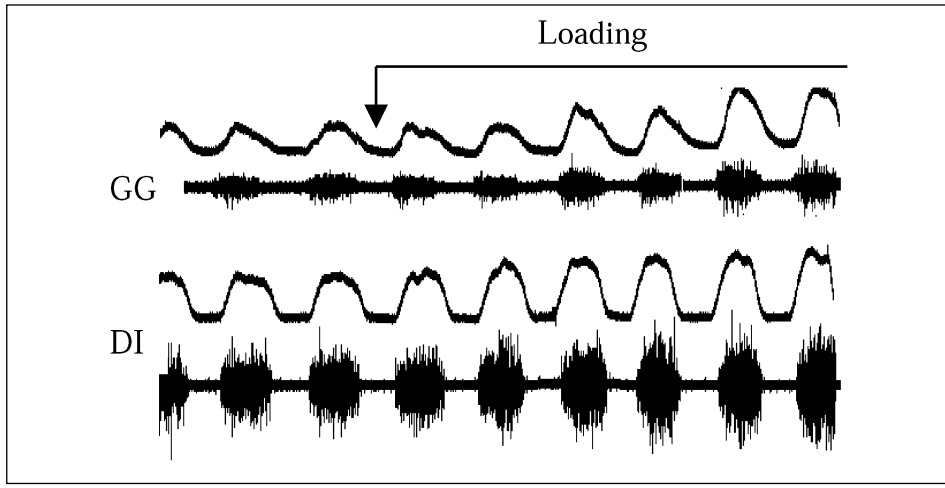

Fig. 2. EMG responses of the genioglossus (GG) and diaphragm (DI) muscles to inspiratory resistive loading in a vagatomized rabbit.

It is known that the increase in diaphragm activity in the first loaded breath is mediated by a decrease in inhibitory afferent activity arising from pulmonary stretch receptors (smaller tidal volume during loaded breathing) (18). In the present study, inspiratory resistive load reduced tidal volume to 60% of its baseline value, which must have weakened vagal volume-related feedback. On

the basis of these data, it may be supposed, by analogy to the diaphragm, that the genioglossus recruitment in the first loaded effort resulted from a decrease in the inhibitory input from pulmonary stretch receptors. The findings obtained in the vagotomized animals, as outline below, confirmed that point of view.

We observed, as did other investigators (5, 9, 15, 16, 19), a markedly stimulating effect of vagotomy on the genioglossus. Section of the vagus nerves abolished the volume-related feedback (Hering-Breuer inflation-inhibition reflex) and evoked an increase in magnitude and duration of inspiratory discharges of both the genioglossus and the diaphragm. It should be noted that the effect of vagotomy on the genioglossus activity was much more than that on the diaphragm activity. After vagotomy, the peak genioglossus EMG activity rose to 316 ±35%, whereas this value for the diaphragm increased to 131 ±12% ($P<0.01$).

The inspiratory resistive load did not evoke an increase in duration of genioglossus and diaphragm inspiratory discharges in vagotomized animals. In addition, the peak diaphragm and genioglossus EMG activities did not rise in the first loaded inspiration. The peak value of genioglossus activity increased significantly over the control unloaded breath only in the third loaded inspiration; the increase being likely mediated through the chemoreceptor stimulation (*Fig. 2*).

Thus, our data indicate that, similarly to the diaphragm, the rapid involvement of the genioglossus muscle in the load compensatory response is mediated by the vagal volume-related feedback and results probably from a decrease in inhibitory input to the hypoglossal motoneurons from pulmonary stretch receptors. Reduction of the inhibitory afferent traffic prolongs inspiration and increases the duration of the genioglossus inspiratory discharges, which allows progressing the inspiratory activity of the genioglossus muscle to a higher level.

Influence of arterial oxygenation on the genioglossus response to inspiratory resistive load.

During loading, changes in respiratory motor output are mediated through perturbations in different afferent systems. It is known that phasic inspiratory activity of the genioglossus muscle is influenced by hypoxic and hypercapnic chemoreceptor stimulation (8, 9, 10). In the present study, the greatest increment in the genioglossus activity was found at 10 min of loading (200 ±40%) and was probably mediated through the chemoreceptor stimulation. In addition, we observed substantial effects of alterations in the base level of arterial oxygenation on EMG activity and vagally-mediated EMG responses of the genioglossus muscle.

During resting unloaded breathing, genioglossus inspiratory discharges were present only during normoxia or hypoxia ($P_{ET}O_2$ =48±5 mmHg). Hyperoxia ($P_{ET}O_2$ >200 mmHg) suppressed inspiratory activity of the genioglossus muscle. Only could week tonic EMG activity be seen during hyperoxia. The genioglossus EMG response to both vagotomy and inspiratory resistive load during hyperoxia was much less than that during hypoxia and normoxia (*Fig. 3*). Prolonged breathing

with oxygen completely abolished the genioglossus activity during the resting breathing and its responses to vagotomy and loading. Therefore, even removal of one of the strongest inhibitory reflex influences (vagal-volume feedback) on upper airway muscle activity does not result in genioglossus activation in the absence of peripheral chemoreceptor drive. Suppression of genioglossus EMG responses by hyperoxia probably results from decreased peripheral chemoreceptor afferent input running to the hypoglossal motoneurons, because hyperoxia decreases the afferent output from peripheral chemoreceptors (19). Breathing with hypoxic gas mixture restored the respiratory activity of the genioglossus and its responses to resistive load in the same animal. In contrast, there were no significant differences in EMG responses of the diaphragm to loading during hypoxia and hyperoxia. Thus, our data demonstrate that peripheral chemoreceptor drive markedly modulated vagal volume-related influences on the genioglossus muscle and that the level of arterial oxygenation before loading was an important determinant of the immediate genioglossus response to added mechanical load.

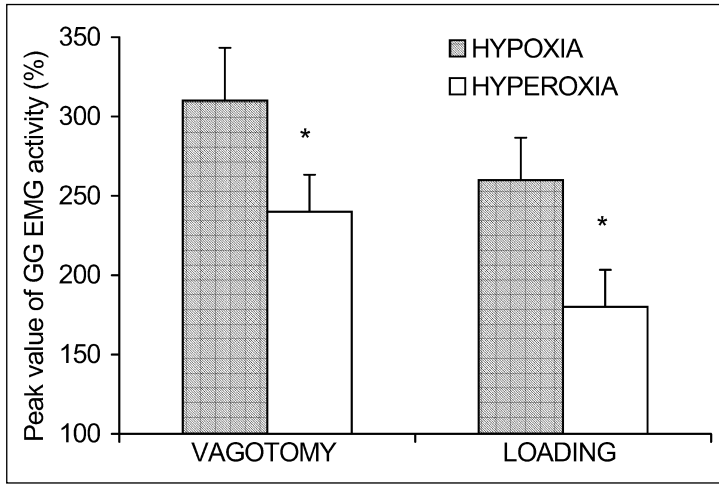

Fig. 3. Magnitude of the genioglossus (GG) responses to vagotomy and to maximal resistive loading (in the first loaded breath) in hypoxic and hyperoxic conditions. *P<0.05 between the responses in either conditions.

In conclusion, the involvement of the pharynx dilating muscles in the load compensatory response appears to be the result of a complex integration of several influences originating from mechanoreceptors and chemoreceptors. The vagal volume-related feedback and peripheral chemoreceptor drive are primarily responsible for the genioglossus response to inspiratory resistive load. We believe that the vagal volume-related feedback is required for the immediately genioglossus activation and the reestablishment of upper airway patency in response to acute obstruction. Chemoreceptor activation is more important for the long-term response of the pharynx dilating muscles to increased resistance to breathing.

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