

The Reflex Effects on the Respiratory Regulation of the CO₂ at the Different Flow Rate and Concentration

Nermin Yelmen, Gulderen Sahin, Tulin Oruc, and Ibrahim Guner

Department of Physiology, Istanbul University, Cerrahpaşa Medical School, Istanbul, Turkey.

Purpose: The purpose of this study was to investigate the activation of the respiratory centers during insufflation of the larynx with CO₂ at different flow rates and concentrations.

Materials and Methods: The experiments were carried out in spontaneous air breathing rabbits, anesthetized with thiopental sodium (25 mg kg⁻¹ i.v.). The larynx was separated from the oropharyngeal cavity and the trachea. The tidal volume (V_T) and respiratory frequency (f min⁻¹) were recorded from the lower tracheal cannula. The respiratory minute volume (V_E) was calculated, the action potentials from the right phrenic nerve were recorded and the inspiratory (T_I) and expiratory (T_E) periods and the mean inspiratory flow rate (V_T/T_I) were calculated. The larynx was insufflated at flow rates of 500 mL min⁻¹ and 750 mL min⁻¹, with 7 and 12% CO₂-Air by means of a respiratory pump. **Results:** Insufflation of the larynx, with both gas mixtures, decreased the f and V_T significantly. The T_I and T_E were found to increase significantly due to the decreasing in f. There was a significant decrease in V_T/T_I ratio. Following bilateral midcervical vagotomy, on the passing of both gas mixtures, significant decreases were observed in the V_T, and the responses of f, T_I and T_E were abolished. After cutting the superior laryngeal nerve, the responses of the V_T to both gas mixtures were abolished. **Conclusion:** In conclusion, the results of this study purpose that the stimulation of the laryngeal mechanoreceptors by the effect of hypercapnia decreases the activation of the respiratory center.

Key Words: Laryngeal mechanoreceptors, hypercapnia, control of breathing

INTRODUCTION

Many investigators have shown histologically that free neural plexuses, with or without myelin,

could exist in the laryngeal mucosa and sub-mucosa.¹⁻⁵ Various classifications have been set forth with regard to the sensory receptors localized in the larynx. Based on the studies of San't Ambrogio et al.⁶ it is assumed that there are 5 different types of receptor in the larynx; these being pressure, drive, cold, irritant and C-fiber receptors.

It has been shown that the laryngeal receptors are sensitive to a wide range of physical and chemical stimulants, that various gases and aerosols stimulate the laryngeal receptors, which has also been confirmed by the potential records from the superior laryngeal nerve (SLN). Likewise, administration of CO₂ at a constant flow rate through the larynx on dogs,⁷ cats⁸ and decerebrated cats⁹ under anesthesia stimulates the laryngeal mechanoreceptors.

It is reported that the laryngeal mechanoreceptors are sensitive to air flow as well as hypercapnic gas and reduced ventilation.^{1,8,10} In studies conducted on cat, it has been shown that constant CO₂ administration to the upper airway inhibited respiration as a reflex and increased the motor neural activity of the upper airway muscle.^{11,12} Furthermore, constant pressure and/or air flow in the isolated upper airway has been shown to influence the tonus of the intrinsic laryngeal muscles and modulate the phasic respiratory activity.¹³

It is also suggested that the hypercapnia caused further increase in the width of the glottis for reducing inspiratory resistance, with its further narrowing during expiration, in turn preventing alveolar collapse.¹⁴ On the other hand, upper airway CO₂ also enhances the laryngeal negative-pressure activity during upper airway occlusion.

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Reprint address: requests to Dr. Nermin Yelmen, Department of Physiology, Istanbul University, Cerrahpaşa Medical School, 34098 Fatih-Istanbul, Turkey. Tel: 90-212-4143071, Fax: 90-210-4143072, E-mail: nermink@istanbul.edu.tr

This is why the activity of negative-pressure receptors is also dependent on the levels of CO₂ occurring in the upper airway during inspiration and expiration.¹⁵ Stimulation of the laryngeal receptors by CO₂ and flow changes and afferent laryngeal impulses may have important roles in the regulation of respiration and breathing patterns under pathophysiological conditions.

The laryngeal lumen is known to be physically subjected to CO₂, but only during expiration. Particularly under conditions where the flow rate varies, for example in the cases of hyperventilation or dyspnea, this variation or the end-tidal CO₂ may influence respiration through the laryngeal receptors.

The aims of this study were to investigate whether the flow rate and concentration of the gas passing through the larynx has an influence upon the respiratory response to CO₂ passage through the larynx and also if the ambient air flow rate would impose a change on the respiratory response.

For these purposes, along with the respiratory reflex responses occurring in the passage of the hypercapnic gas mixtures (7% CO₂-Air, 12% CO₂-Air), the central inspiratory activity and variations in the inspiration and expiration were also sought from recording of the phrenic neural action potential.

MATERIALS AND METHODS

In our study, 9 New Zealand type Albino rabbits of both sexes (mean body weight 2.8 ± 0.75 kg) were anesthetized using thiopental sodium (25 mg.kg⁻¹ i.v.). Following a lower cervical tracheotomy, a cannula caudally connected to the inspiration-expiration valve was placed in the trachea. The test animal was made to respire through that cannula. The pharynx hyoid was then widely opened on the right rostral side of the bone, and the epiglottis slowly pulled ventrally.

To the lower part of the cricoid cartilage, a second tracheal cannula was rostrally mounted. During these operations, utmost care was taken to avoid disturbance of the afferent (superior laryngeal nerve) and efferent (recurrent laryngeal nerve) nerves.⁴ The right-hand and left-hand a.

femoralis and v. femoralis were isolated, and a catheter placed therein. To prevent any thrombosis or clogging of the catheters during the operation, the test animals were injected i.v. with liquiemin (0.2 mL kg⁻¹).

Through the larynxes of the test animals, two different hypercapnic gas mixture concentrations, namely ambient air (22°C) and 7% CO₂-Air and 12% CO₂-Air, were supplied at rates of 500 mL/min and 750 mL/min. In the meantime, the experimental animals constantly respired normoxic ambient air through the lower tracheal cannula. Furthermore, a tracheotomy was performed and the tracheal cannula, connected to a inspiratory-expiratory valve, was inserted into the trachea. The tidal volume (V_T) and respiratory frequency (f min⁻¹) were recorded on a Grass Model 7 polygraph by means of a pneumatograph and a Grass PT-5 volumetric pressure transducer. From these parameters, the respiratory minute volume (V_E) was calculated. From the right-hand a. femoralis, the systemic arterial blood pressure was recorded using a physiological pressure transducer, and the mean systemic arterial pressure calculated.

A nerve to the right side was isolated in order to record action potential from the N. Phrenicus. Under a stereomicroscope, the nerve was cut at the lower 1/3 part of the N. phrenicus neck zone at the point where the branches coming from the 5th and 6th cervical roots meet. The sleeve of the N. phrenicus, originating from the 5th root, was stripped off under microscopy. After this, the nerve was placed on a platinum electrode. In order that the nerve would preserve its activity through the testing period, the above mentioned region was stored in neutral paraffin. The electrodes were connected to the 7 P5 preamplifier of the Grass model 7 polygraph in an effort to record the action potential from the N. phrenicus. From the discharge group period of the N. phrenicus action potentials, the inspiration period (T_I) was determined, while from the periods between the discharge groups the expiration period (T_E) was determined. The tidal volume (V_T) was divided by the inspiration period (T_I) to give the mean inspiratory flow rate (V_T/T_I).

At the end of each phase, the PaO₂, PaCO₂ and pH_a were determined from the blood samples

received from the left femoral artery, using the AVL gas check type 937 at 37°C.

Experimental procedure

Pump is open (at the rates of 500 mL/min and 750 mL/min)

15 minutes, normoxic air passage through larynx

5 minutes, 7% CO₂-Air passage

15 minutes, normoxic air passage

5 minutes 12% CO₂-Air passage

Statistical analysis

The statistical significance of the changes in the respiratory parameters before and after administration of the two hypercapnic gas mixtures was tested with a Wilcoxon Matched Pairs test.

RESULTS

The influences of the normoxic phase

To distinguish the influences of the air flow and hypercapnic gas mixtures on the larynx of the test animals, ambient air was first passed at the defined by means of the respiratory pump. However, no variation was observed in the f (min⁻¹), and the V_T first decreased, but then reached its normal

value. A decreased V_T response was observed for only a few seconds (10 to 15 seconds). No meaningful variation was observed in the V_E . Also, no meaningful variations were observed in the T_I and T_E periods. After a bilateral vagotomy, there were no changes in the respiratory parameters (Table 1).

While the pump speed was 500 mL min⁻¹ The influences of 7% CO₂-Air

When 7% CO₂-Air was passed through the larynx of the test animals at the given pump speed, significant reductions in both the V_T and f (min⁻¹) were observed. Subject to the variation in the V_T and f (min⁻¹), the V_E also realized a significant reduction. In accordance with the frequency reduction, significant extensions were observed in the T_I and T_E values, while the V_T/T_I rate revealed a significant reduction (Table 2).

After a bilateral midcervical vagotomy, on passing 7% CO₂-Air through the larynx, a significant decrease was again observed in the V_T , as seen in the control group. In contrast, the reduction observed in the frequency was fully eliminated. Also, no significant variation was seen in the T_I and T_E periods, but the V_T/T_I realized a significant reduction (Table 2).

Following a bilateral vagotomy and bilateral cutting of the SLN, the 7% CO₂-Air mixture was passed, and the reduction previously observed in the V_T was completely eliminated, although no

Table 1. Effects of Ambient Air at 500 mL min⁻¹ and 750 mL min⁻¹ Passing through the Larynx on the Respiratory Parameters in the Indicated Experimental Phases (means ± SE)

| | n = 9 | f (min ⁻¹) | V _T (mL) | V _E (mL min ⁻¹) | T _I (s) | T _E (s) |
|----------|--------------|------------------------|---------------------|--|--------------------|--------------------|
| Control | Air | 61.6 ± 0.9 | 23.6 ± 0.8 | 1455.7 ± 158.2 | 0.48 ± 0.02 | 0.49 ± 0.02 |
| | Pump 1 + Air | 61.6 ± 0.7 | 20.8 ± 1.7 | 1392.9 ± 156.9 | 0.48 ± 0.01 | 0.49 ± 0.01 |
| | Air | 54.0 ± 1.7 | 26.5 ± 2.3 | 1429.6 ± 88.2 | 0.53 ± 0.01 | 0.57 ± 0.01 |
| | Pump 2 + Air | 53.8 ± 5.8 | 25.6 ± 3.1 | 1359.8 ± 123.2 | 0.54 ± 0.01 | 0.57 ± 0.05 |
| Vagi cut | Air | 28.0 ± 1.8 | 47.3 ± 9.2 | 1324.4 ± 88.9 | 1.03 ± 0.03 | 1.11 ± 0.03 |
| | Pump 1 + Air | 27.9 ± 2.01 | 45.5 ± 8.3 | 1181.5 ± 120.2 | 1.03 ± 0.04 | 1.11 ± 0.03 |
| | Air | 35.3 ± 4.2 | 41.5 ± 2 | 1464.9 ± 116.5 | 0.84 ± 0.02 | 0.85 ± 0.03 |
| | Pump 2 + Air | 35.0 ± 4.1 | 40.2 ± 1.6 | 1411.8 ± 89.1 | 0.84 ± 0.04 | 0.86 ± 0.05 |

f , respiratory frequency; V_T , tidal volume; V_E , respiratory minute volume; T_I , inspiratory period; T_E , expiratory period.
Pump 1 rate: 500 mL min⁻¹, Pump 2 rate: 750 mL min⁻¹.

Table 2. Effects of 7 and 12% CO₂-Air Mixtures Passing through the Larynx on the Respiratory Parameters in the Indicated Experimental Phases (pump rate 500 mL min⁻¹) (means ± SE)

| | n = 9 | f (min ⁻¹) | V _T (mL) | V _E (mL min ⁻¹) | T _I (s) | T _E (s) | V _T /T _I (mL s ⁻¹) |
|--------------------------|--------------------------|-------------------------|-------------------------|--|--------------------|--------------------------|--|
| Control | Air | 48.9 ± 1.9 | 39.8 ± 2.6 | 1935.9 ± 148.3 | 0.59 ± 0.08 | 0.63 ± 0.12 | 67.4 ± 8.2 |
| | 7% CO ₂ -Air | 45.1 ± 2.0 [‡] | 31.7 ± 2.2 [‡] | 1450.1 ± 132.1 [‡] | 0.62 ± 0.10* | 0.72 ± 0.13 [†] | 51.1 ± 5.3* |
| | Air | 51.8 ± 3.7 | 33.9 ± 2.7 | 1712.4 ± 123 | 0.56 ± 0.09 | 0.59 ± 0.10 | 59.6 ± 6.1 |
| | 12% CO ₂ -Air | 47.5 ± 3.4 [†] | 26.1 ± 2.5 [†] | 1191.9 ± 55 [†] | 0.61 ± 0.12* | 0.64 ± 0.13 [†] | 42.4 ± 2.3 [†] |
| Vagi cut | Air | 39.7 ± 5.7 | 59.2 ± 10.2 | 2350.2 ± 538.8 | 0.74 ± 0.07 | 0.77 ± 0.09 | 80.0 ± 4.2 |
| | 7% CO ₂ -Air | 39.5 ± 5.8 | 50.5 ± 9.1 [‡] | 1994.7 ± 474 [‡] | 0.78 ± 0.10 | 0.77 ± 0.11 | 64.7 ± 3.9 [†] |
| | Air | 40.8 ± 4.0 | 54.1 ± 2.2 | 2207.2 ± 276.3 | 0.72 ± 0.13 | 0.75 ± 0.08 | 75.0 ± 5.2 |
| | 12% CO ₂ -Air | 40.2 ± 4.2 | 43.8 ± 3.2 [†] | 1760.7 ± 203.6 [†] | 0.73 ± 0.09 | 0.76 ± 0.08 | 60.0 ± 4.8* |
| Vagi cut + sln cut | Air | 39.8 ± 5.5 | 52.7 ± 7.8 | 2097.4 ± 154.5 | 0.74 ± 0.11 | 0.76 ± 0.09 | 71.2 ± 4.7 |
| | 7% CO ₂ -Air | 40.5 ± 5.4 | 52.9 ± 7.7 | 2142.4 ± 154.6 | 0.73 ± 0.08 | 0.75 ± 0.10 | 72.4 ± 2.9 |
| | Air | 41.2 ± 2.3 | 53.2 ± 4.2 | 2190.8 ± 205 | 0.70 ± 0.08 | 0.75 ± 0.06 | 75.4 ± 3.8 |
| | 12% CO ₂ -Air | 40.9 ± 3.9 | 53.4 ± 2.9 | 2184 ± 210 | 0.70 ± 0.14 | 0.75 ± 0.13 | 75.6 ± 2.6 |

f, respiratory frequency; V_T, tidal volume; V_E, respiratory minute volume; T_I, inspiratory period; T_E, expiratory period; V_T/T_I, mean inspiratory flow rate.

**p* < 0.05, [†]*p* < 0.01, [‡]*p* < 0.001 for change compared with air phase.

variation was observed in the T_I and T_E periods (Table 2).

The influences of 12% CO₂-Air

When the hypercapnic gas mixture, consisting of 12% CO₂-Air was passed through the larynx, at a pumping rate of 500 mL min⁻¹, significant reductions were observed in the V_T and f (min⁻¹), and therefore in the V_E. Meaningful extensions in T_I and T_E were observed, while the V_T/T_I rate again was significantly reduced (Table 2).

After a bilateral midcervical vagotomy, when 12% CO₂-Air was passed through the larynx, a significant decrease was again observed in the V_T, as seen in the control group. On the other hand, the reduction observed in the frequency was abolished. Therefore, no significant variation was seen in the T_I and T_E periods, but the V_T/T_I realized a significant reduction (Table 2).

After bilateral cutting of the SLN, the reduction in V_T was eliminated, and again no change observed in the T_I and T_E periods (Table 2).

When the pumping speed in Table 4 was 500 mL min⁻¹, the percentage changes were seen

depending on the air phase in the determined parameters on passing of the two hypercapnic gases. When 12 and 7% CO₂-Air were passed through the larynx at the same rate, the percentage change in the reductions observed in the V_T and V_E were more significant with the 12% CO₂-Air compared with the that of the 7% CO₂-Air.

While the pumping speed was 750 mL min⁻¹ The influences of 7% CO₂-Air

When 7% CO₂-Air was passed through the larynx of the test animals at the given pump speed (750 mL/min), again significant reductions were observed in the V_T and f (min⁻¹), and therefore in the V_E. Significant extensions were observed in the T_I and T_E periods, in agreement with the frequency reduction. The V_T/T_I ratio was also significantly decreased (Table 3).

Following a bilateral midcervical vagotomy, there was a significant decrease in the V_T, as seen in the control group. The frequency reduction was completely eliminated, and no changes observed in the T_I and T_E periods. The V_T/T_I ratio again underwent a significant reduction (Table 3). After

Table 3. Effects of 7 and 12% CO₂-Air Mixtures Passing through the Larynx on the Respiratory Parameters in the Indicated Experimental Phases (pump rate 750 mL min⁻¹) (means ± SE)

| | n = 9 | f (min ⁻¹) | V _T (mL) | V _E (mL min ⁻¹) | T _I (s) | T _E (s) | V _T /T _I (mL s ⁻¹) |
|--------------------------|--------------------------|-------------------------|-------------------------|--|--------------------|--------------------------|--|
| Control | Air | 42.0 ± 4.5 | 33.5 ± 5.6 | 1121.4 ± 120.9 | 0.68 ± 0.10 | 0.74 ± 0.09 | 49.5 ± 3.1 |
| | 7% CO ₂ -Air | 38.5 ± 4.0 [†] | 29.9 ± 3.6 [‡] | 1052.0 ± 146.8 [‡] | 0.72 ± 0.07* | 0.82 ± 0.10 [†] | 41.5 ± 4.7* |
| | Air | 52.6 ± 3.9 | 32.8 ± 3.4 | 1665.2 ± 88.3 | 0.55 ± 0.10 | 0.59 ± 0.12 | 59.6 ± 5.5 |
| | 12% CO ₂ -Air | 49.1 ± 3.9 [†] | 23.3 ± 3.4 [†] | 1086.6 ± 88.8 [†] | 0.58 ± 0.09* | 0.63 ± 0.08 [†] | 39.6 ± 2.8 [†] |
| Vagi cut | Air | 33.0 ± 2.2 | 53.0 ± 3.1 | 1749.0 ± 165.7 | 0.85 ± 0.12 | 0.96 ± 0.10 | 62.3 ± 2.6 |
| | 7% CO ₂ -Air | 33.0 ± 2.1 | 45.7 ± 3.0 [‡] | 1508.1 ± 135 [†] | 0.85 ± 0.11 | 0.96 ± 0.09 | 53.8 ± 5.7 [†] |
| | Air | 33.6 ± 1.4 | 56.6 ± 3.8 | 1901.7 ± 236 | 0.83 ± 0.08 | 0.95 ± 0.07 | 68.1 ± 3.0 |
| | 12% CO ₂ -Air | 34.4 ± 1.4 | 45.5 ± 2.9 [†] | 1565.2 ± 190 [‡] | 0.82 ± 0.08 | 0.92 ± 0.06 | 55.4 ± 2.9* |
| Vagi cut + sln cut | Air | 33.8 ± 1.4 | 56.8 ± 4.6 | 1919.8 ± 95.7 | 0.83 ± 0.11 | 0.96 ± 0.10 | 68.4 ± 2.1 |
| | 7% CO ₂ -Air | 33.8 ± 1.3 | 56.9 ± 3.4 | 1923.2 ± 93.5 | 0.83 ± 0.10 | 0.96 ± 0.10 | 68.5 ± 1.7 |
| | Air | 34.5 ± 1.9 | 54.1 ± 5.2 | 1866.4 ± 212 | 0.82 ± 0.12 | 0.91 ± 0.13 | 65.9 ± 3.2 |
| | 12% CO ₂ -Air | 34.5 ± 1.8 | 54.0 ± 5.1 | 1863.0 ± 210 | 0.82 ± 0.12 | 0.91 ± 0.13 | 65.8 ± 4.6 |

f, respiratory frequency; V_T, tidal volume; V_E, respiratory minute volume; T_I, inspiratory period; T_E, expiratory period; V_T/T_I, mean inspiratory flow rate.

**p* < 0.05, [†]*p* < 0.01, [‡]*p* < 0.001 for change compared with air phase.

a vagotomy, by means of bilateral cutting of the SLN, the reduction in V_T was completely eliminated, and no variations observed in the T_I and T_E period (Table 3).

The influences of 12% CO₂-Air

When 12% CO₂-Air was passed through the larynx at the same pumping speed (750 mL min⁻¹), significant reductions were observed in the f (min⁻¹) V_T and V_E. The extensions in T_I and T_E were also found to be significant. A significant reduction was observed in the V_T/T_I ratio (Table 3).

A bilateral midcervical vagotomy again produced a significant decrease in the V_T and V_T/T_I ratio and eliminated the reduction in f (min⁻¹). No meaningful variation was observed in the T_I and T_E periods.

After a vagotomy and bilateral cutting of the SLN, the reduction in the V_T formed in response to hypercapnia was completely eliminated. No significant variation was observed in the T_I and T_E periods (Table 3).

To see the influence of differing CO₂ concentra-

tions on the parameters at the same pumping speed, the percentages in the variations were calculated (Table 4). When a hypercapnic gas mixture, consisting of 12% CO₂-Air, was passed through the larynx at a pump rate of 750 m/min, the percentage changes in the reduction observed in the V_T and V_E were much higher with the 12 than the 7% CO₂-Air mixture (Table 4).

On passing both hypercapnic gas mixtures through the larynx, significant changes in the PaO₂, PaCO₂ and pH_a values were not observed (Table 5).

DISCUSSION

The laryngeal mechanoreceptors are sensitive to pressure and/or flow, with rapid or slow adapting response patterns.^{1,2} They generally increase their activities through inspiration in response to the respiratory movements of the larynx.¹⁶ Intralaryngeal CO₂ reduces ventilation as a reflex and increases the upper airway muscular activity.^{1,8-10,16-19}

In our study, before passing the gas mixtures

Table 4. The Mean Percentage Variations in the Respiratory Parameters of the Control Group Compared with the Air Phase in the Indicated Experimental Phases (means \pm SE)

| n = 9 | f (min ⁻¹) | V _T (mL) | \dot{V}_E (mL min ⁻¹) | T _I (s) | T _E (s) | V _T /T _I (mL s ⁻¹) |
|------------------------------------|------------------------|---------------------|-------------------------------------|--------------------|--------------------|--|
| Pump rate 500 mL min ⁻¹ | | | | | | |
| 7% CO ₂ -Air | -7.8 \pm 2.6 | -20.3 \pm 3.6 | -25.1 \pm 6.5 | +5.0 \pm 0.8 | +14.2 \pm 0.5 | -9.4 \pm 2.3 |
| 12% CO ₂ -Air | -8.3 \pm 2.4 | -23.0 \pm 4.6* | -30.4 \pm 7.4* | +8.2 \pm 0.5 | +9.3 \pm 0.8 | -28.8 \pm 3.2* |
| Pump rate 750 mL min ⁻¹ | | | | | | |
| 7% CO ₂ -Air | -8.3 \pm 1.5 | -10.7 \pm 4.2 | -6.1 \pm 3.5 | +6.4 \pm 0.8 | +11.4 \pm 0.6 | -5.5 \pm 1.8 |
| 12% CO ₂ -Air | -6.6 \pm 1.7 | -28.9 \pm 3.5* | -34.7 \pm 3.2* | +6.7 \pm 0.8 | +7.2 \pm 0.6 | -33.5 \pm 3.8* |

f, respiratory frequency; V_T, tidal volume; \dot{V}_E , respiratory minute volume; T_I, inspiratory period; T_E, expiratory period; V_T/T_I, mean inspiratory flow rate.

*Indicates significance between the response to both hypercapnic gas mixtures (**p* < 0.05)

Table 5. Values of PaO₂, PaCO₂ and pH_a of Rabbits in the Indicated Experimental Phases (means \pm SE)

| Experimental phase (n = 9) | PaO ₂ (mmHg) | PaCO ₂ (mmHg) | pH _a |
|----------------------------|-------------------------|--------------------------|------------------|
| Air | 89.3 \pm 3.1 | 38.4 \pm 2.5 | 7.36 \pm 0.002 |
| 7% CO ₂ -Air | 87.9 \pm 4.7 | 39.0 \pm 2.9 | 7.35 \pm 0.002 |
| Air | 88.2 \pm 4.3 | 38.9 \pm 2.7 | 7.35 \pm 0.001 |
| 12% CO ₂ -Air | 88.9 \pm 6.2 | 38.5 \pm 2.9 | 7.36 \pm 0.001 |

consisting of 7% and 12% CO₂-Air directly through the larynx, 500 mL min⁻¹ and 750 mL min⁻¹ ambient air were passed for the purpose of determining the influence of the air flow on ventilation. In doing so, the influences of the air flow and hypercapnic gas mixtures on the larynx were distinguished. As seen from our findings, when ambient air was passed through the larynx, the reduction in the tidal volume recovered to its normal value in a short time. No significant variation was observed in the respiration frequency. While the bilateral vagotomy did not influence the short time reduction in the V_T, cutting the SLNs bilaterally eliminated this response. As no short time reduction was observed in the V_T by means of cutting the afferent nerves of the larynx, this is evidence that the response is through the laryngeal mechanoreceptors. Likewise, in rabbits, it is observed that the laryngeal mechanosensitive endings increased their discharges through stress, while such discharges were reduced by collapse.^{18,20} Our finding shows that the mechanoreceptors adapt the ambient air passage very quickly.

In our study, the T_I and T_E periods were observed to be extended, with reductions in the respiration frequency and volume while passing both hypercapnic gases through the larynx at a rate of 500 mL min⁻¹. When the mean inspiratory flow rate (V_T/T_I) was calculated, this value was considerably decreased when passing both hypercapnic gases through the larynx. In such a case, both the hypercapnic gas mixtures can be said to have passed through the larynx, which inhibit the ventilation by means of reducing the central inspiratory activity. Because the inspiration period gets longer, the central inspiratory activity takes a long time, but as the rising rate is low, it can reach a relatively lower value. The reduced tidal volume also supports this finding.

Boushey et al.⁸ administered three different concentrations, namely 5%, 10% and 30% CO₂-Air mixtures, to the anesthetized and decerebrated cats at the larynx with a syringe, and observed that 5 and 10% CO₂ reduced the minute volume by only slowing down the respiration. A biphasic response occurred against 30% CO₂, first the

respiration frequency and volume reduced, and after 2 to 10 respiratory cycles, the tidal volume was restored to its control level, although the respiratory frequency remained lower.

In our finding, the reductions in ventilation when passing 7 and 12% CO₂ were both associated with the reductions in the tidal volume and respiratory frequency.

In other studies, it has been demonstrated that ipsilateral SLN stimulation caused an interruption of the activity in the dorsal respiratory group neurons for a short time.²¹⁻²³ Furthermore, following the SLN stimulation, it has been reported that inhibitor responses were formed on the soma of inspiratory laryngeal motor neuron, while excitator responses occurred on the axon.²¹ SLN stimulation is reported to have strong inhibitions on phrenic neural discharge and hyperpolarization on the phrenic motor neural membrane potentials.^{24,25} In light of these data, the reduction in the central inspiratory activity is due to the influence of the direct laryngeal afferent impulses on the respiration centers.

In our findings, it was surprising that the respiratory frequency did not change in passing both hypercapnic gas mixtures through the larynx following the bilateral cervical vagotomy. The vagotomy eliminated the inhibitory effect of CO₂ on the respiration frequency. A cervical vagotomy also eliminated the efferent impulses that would reach the larynx through the laryngeal nerve. Van Lunteren et al.²⁶ set forth that the activity of the upper airways was modulated by a feedback arranged through vagal means, which had a greater value than that of the phrenic nerve and diaphragm. Apparently, the vagal fibers are very significant for upper airway modulation and adjustment of the respiratory rhythm.²⁷ It can be thought that its mechanoreceptors had a vagal innervation, which are stimulated by CO₂ and thereby affected the frequency.

On the other hand, after cutting the afferent nerves of the larynx following a vagotomy, the variation in the respiratory volume was also eliminated, which shows that the receptors stimulated by CO₂ and inhibiting the VT had sent their impulses to the center through the superior laryngeal nerves.^{1,2}

Bradford et al.¹⁵ have shown that stimulation of

the laryngeal receptors by 5 and 9% CO₂ caused reflex influences on the ventilation and upper airway muscular activity, but there was no significant difference between the responses caused by the constant administration of the differing concentrations of CO₂ or during expiration. Our findings show that the increasing hypercapnic gas concentration, passing at the same flow rate, particularly support the reduction in the V_T; and therefore in the V_E. When the mean inspiratory flow rates of the 7 and 12% CO₂-Air mixtures were compared, the decrease was greater with the 12% CO₂-Air mixture. So far, no study has realized how the mechanoreceptors are stimulated by CO₂. However, it can be said that the greater the CO₂ concentration, the less the central inspiratory activity.

Stella et al.²⁸ studied the influence of negative pressure, inspiration and the expiratory flow at positive pressure and expiration on the airway of the inspiratory flow. These investigations have shown that the tidal volume and diaphragmatic and abdominal muscular activities were not affected by the upper airway flow and/or pressure at ambient and body temperatures, and that the total respiratory time and expiration time had very minor increases.

In our study, the respiratory frequency decreased due to the significant increases in the T_I and T_E periods, as estimated from the direct phrenic activity. Therefore, it can be said that the intralaryngeal CO₂ influenced the respiratory rhythm through the organization of the laryngeal receptors.

In their study, Bartlett et al.⁹ recorded the single fiber afferent activity from the superior laryngeal nerve when 3.5 and 10% CO₂ were passed at a constant flow rate in anesthetized and paralyzed cats. They set forth that the discharge frequency of 48 out of the 53 receptors were decreased by increasing CO₂ concentrations. They also showed that only 5 receptors were stimulated by intralaryngeal CO₂. As a result, they stated that the reflex responses caused by CO₂ were associated with the weakening of the laryngeal receptor activity and the variation in the afferent impulse discharge that commence from the larynx due to ventilatory inhibition.

It is a well known fact that in rabbits the

laryngeal mechanosensitive endings change their discharges through negative and positive pressures^{1,29} Therefore, when the pumping speed was increased in order to determine the relation between the straining of the larynx and the concentration of the hypercapnic gas passing through, it was seen that at low hypercapnic gas concentrations, increasing the flow rate reduced the ventilation inhibition, but conversely, under higher CO₂ concentrations, increasing the flow rate in turn increased the ventilation inhibition.

Apparently, the greater the flow rate, the less the unit time needed for lower concentrations of hypercapnic gas to affect the receptors. Besides, the influence in the unit time gets stronger with increasing concentration.

According to our findings, the air passage CO₂ concentration and flow changes play important roles in the reflex regulation of ventilation by stimulating the laryngeal mechanoreceptors. Under the pathophysiology conditions of the upper airways, and in the application of non-invasive mechanical ventilation, stimulation of the laryngeal afferents can produce important reflex effects on the respiratory regulation and upper airway patency. In fact, during non-invasive mechanical ventilation (NIMV) the laryngeal mechanoreceptors could be stimulated by the affect of flow and CO₂ concentration changes. With NIMV the ventilator forces air through the vocal cords, which are well known to widen during spontaneous inspiration. During NIMV the substantial turbulence occurring in this area strongly stimulates the laryngeal mechanoreceptors and decreases ventilation. As a matter of fact, apnea could occur following mechanical ventilation due to the affect of the flow.³⁰

Our findings show that local laryngeal hypercapnia inhibits the central inspiratory activity by means of a reflex pathway; and furthermore, the increases in both the hypercapnic gas concentration and in the passage speed, produce stronger reflex inhibition in the ventilation. It is for this reason that, although there may be other factors involved, our findings indicate that the inhibition of the central inspiratory activity caused by the increase in both the hypercapnic gas concentration and the flow rate may play a role in apnea after NIMV.

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