

Modification of Laryngospasm in Response to Changes in Pa_{CO_2} and Pa_{O_2} in the Cat

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In order to study the effects of Pa_{CO_2} and Pa_{O_2} on the laryngeal closure reflex, changes in laryngeal resistance of the isolated cat larynx were measured before and during the stimulation of the superior laryngeal nerve (SLN) at various levels of Pa_{CO_2} and Pa_{O_2} . The results showed that laryngeal resistance before SLN stimulation [$L_{(th\text{base})}$] increased slightly during hypocapnia. SLN stimulation produced laryngospasm which was defined as a sharp rise in the laryngeal resistance. Hypercapnia alone and hypoxia alone increased ventilation but decreased the degree and duration of laryngospasm due to SLN stimulation. On the other hand, hypocapnia augmented and prolonged the duration of this laryngospasm. These results suggest that Pa_{CO_2} and Pa_{O_2} regulate the laryngeal closure reflex in a way such that the degree of laryngospasm changes in inverse proportion to the activity of the respiratory center. (Key words: Carbon dioxide; hypercarbia; hypocarbia. Hypoxia. Larynx: spasm.)

THE MAIN FUNCTION of the laryngeal closure reflex is to prevent the entrance of foreign material into the lower respiratory tract.¹ Exaggeration of this reflex may cause laryngospasm, a potential hazard in anesthesia. Since an inadequate level of anesthesia frequently precipitates laryngospasm,² it has been considered that the state of the nervous system plays an important role in the occurrence of laryngospasm.³ Although there have been many studies on laryngospasm,³⁻⁶ there is little information about the effects of chemical ventilatory drive factors such as Pa_{O_2} and Pa_{CO_2} . Since it is known that the laryngeal reflex exerts a dominant influence over the respiratory center⁷ and that the activity of the respiratory center is influenced by Pa_{O_2} and Pa_{CO_2} ,⁸ it is possible that these chemical factors exert some influence over the reflex control of the laryngeal glottis. In order to investigate this possibility, we induced laryngospasm in cats by electrical stimulation of the superior laryngeal nerve at various levels of Pa_{O_2} and Pa_{CO_2} and examined the effects on the production of laryngospasm.

Methods

Ten adult cats weighing 2.5-3.8 kg were anesthetized with α -chloralose (50 mg/kg, ip). Both femoral arteries were catheterized for the measurement of arterial blood pressure and the withdrawal of arterial blood samples. The right femoral vein was also catheterized. Through a vertical midline incision, the larynx and trachea were exposed after removal of the strap muscles. The internal branch of the right superior laryngeal nerve (SNL) was identified and sectioned near its entrance into the larynx, and the central end was placed onto bipolar platinum electrodes in a warm mineral oil bath. At appropriate times during the experiment, rectangular wave stimuli of 0.5 to 2.0 volt, 0.1 ms pulse duration at a frequency of 10 Hz, were delivered to the SLN as stimulus trains in order to produce laryngeal spasm.⁴ The strength of the stimulus was varied from animal to animal, but it was fixed at the minimum intensity level required to cause apnea with adduction of vocal cords for each animal during room air breathing and not varied throughout the experiment.

In order to measure changes in laryngeal resistance during laryngeal spasm, we employed the double tracheal cannulation technique described by Stransky *et al.*⁹ Thus, a caudally directed cannula was tied in the lower cervical trachea and the animal breathed through this cannula (fig. 1). A second tracheal cannula was tied in the upper part of the trachea and was directed rostrally. Care was taken not to damage the recurrent laryngeal nerves during the operation. The pharynx was opened widely in the midline and the epiglottis was gently pulled ventrally with a suture, thus permitting direct visualization of the glottis. A constant stream of humidified warm air passed upward from the upper cannula through the larynx at a constant rate ($0.4 \text{ l} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$), *i.e.*, about 1.0-1.5 l/min, for each animal. The inflow pressure (the upper tracheal pressure) was recorded and the ratio of this inflow pressure to flow rate was used as a measure of laryngeal resistance. Ventilatory airflow was measured through a Fleish pneumotachograph (#00) attached to the lower tracheal cannula and the tidal volume was obtained by electrical integration of inspired airflow. End-tidal CO_2 (P_{ETCO_2}) was measured continuously using a CO_2 analyzer (Beckman® LB-1). All variables

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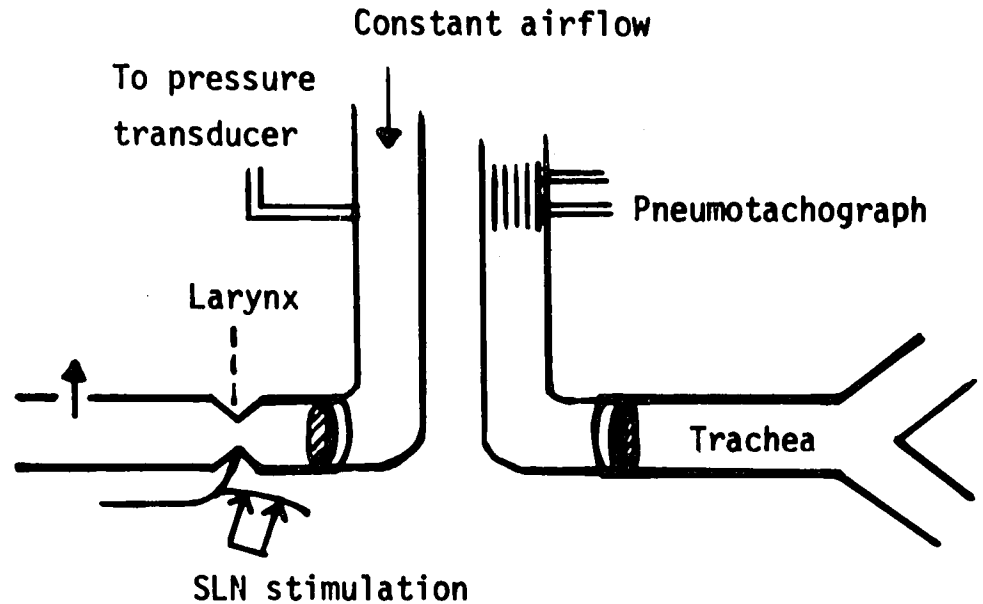


FIG. 1. Schematic illustration of the experimental apparatus. For description, see text.

were recorded on a visicorder (San-ei). The rectal temperature of the animals was maintained close to 38°C with a heating lamp. Arterial blood samples were analyzed for pH, P_{CO₂} and P_{O₂} immediately after sampling using a Radiometer blood-gas analysis system (BMS MK2).

In order to obtain various levels of Pa_{O₂} and Pa_{CO₂}, the inspired gas was controlled using various concentrations of CO₂ in oxygen or various concentrations of O₂ in nitrogen either while the animal was breathing spontaneously or was hyperventilated artificially with a Harvard® respirator. After a change in Pa_{CO₂} or Pa_{O₂}, 5–7 min were allowed to achieve a steady state. Following arterial blood sampling at each steady state level of Pa_{O₂} and Pa_{CO₂}, the SLN was stimulated electrically for 15 s as described above and the evoked changes in laryngeal resistance which represent the degree of laryngeal spasm were calculated from the recordings. Although the study under 100 per cent O₂ breathing was always performed first, random order was followed for the other conditions in each experiment. Concerning blood-gas values, it should be remembered that normal blood-gas values in a cat are slightly different from those of humans.^{10,11} Statistical analysis was performed by using Student's paired *t* test.

Results

EFFECT OF CO₂ AND O₂ ON BASELINE LARYNGEAL RESISTANCE

The effects of CO₂ and O₂ on the mean laryngeal resistance before SLN stimulation [*L_R(baseline)*] are

shown in table 1. The mean value of Pa_{CO₂} when the animals were breathing 100 per cent O₂ was 32.5 ± 0.7 torr, and this was defined as normocapnia in this study. By directly watching the respiratory movement of the vocal cords we observed that hypercapnia alone and hypoxia alone increased the size of the glottis, whereas hypocapnia decreased it. A significant increase in the value of *L_R(baseline)* (*P* < 0.01) was observed during hypocapnia when compared with the value obtained during normocapnia. Although hypoxia and hypercapnia increased the size of the glottis, no measurable change in *L_R(baseline)* was observed.

EFFECTS OF CO₂ AND O₂ ON LARYNGOSPASM BY SLN STIMULATION

Figure 2 shows changes in the upper tracheal pressure and in respiration caused by SLN stimulation at three different levels of Pa_{CO₂} in a single cat. Before SLN stimulation the animal was breathing spontaneously during normocapnia and hypercapnia. Hypocapnia was produced by mechanical hyperventilation, but the pump was stopped at the start of the SLN stimulation in order to eliminate a possible reflex effect of the passive respiratory movement. During normocapnia, spontaneous respiration arrested at the expiratory phase immediately following the start of the SLN stimulation and the subsequent increase in the upper tracheal pressure attained a plateau level during the course of SLN stimulation. During hypercapnia, the SLN stimulation caused apnea but the increase in the upper tracheal pressure was small, indicating that the constriction of the glottis

TABLE 1. Effects of CO₂ and O₂ on L_{R(baseline)}

Pa _{CO₂} (torr)	26.5 ± 1.4	32.5 ± 0.7	42.8 ± 1.0	52.4 ± 1.2	32.5 ± 0.7	32.5 ± 0.7	32.5 ± 0.7	26.5 ± 0.7
Pa _{O₂} (torr)	>350	>350	>350	>350	102.1 ± 2.4	68.0 ± 2.2	51.6 ± 1.7	51.6 ± 1.7
L _{R(baseline)} [cm H ₂ O/(l/min)]	0.360 ± 0.040	0.010 ± 0.003	0.009 ± 0.004	0.008 ± 0.004	0.010 ± 0.003	0.008 ± 0.004	0.008 ± 0.005	0.010 ± 0.004
Mode of ventilation	mechanical	spontaneous	spontaneous	spontaneous	spontaneous	spontaneous	spontaneous	spontaneous

Values are means ± SE, n = 10.

was relatively weak. During hypocapnia, on the other hand, there was a remarkable increase in the upper tracheal pressure, indicating a strong laryngospasm. The relationship between Pa_{CO₂} and the laryngeal resistance at the plateau level of the increased upper tracheal pressure [L_{R(plateau)}] in all animals is shown in figure 3. The values of the L_{R(plateau)} during hypocapnia and hypercapnia were significantly different ($P < 0.01$) from the value of the L_{R(plateau)} during normocapnia. Figure 4 depicts the relationship between Pa_{O₂} and L_{R(plateau)}. As shown, the L_{R(plateau)} decreased progressively with decreasing levels of Pa_{O₂} at a constant Pa_{CO₂}. The value of the L_{R(plateau)} during hyperoxia (Pa_{O₂} > 350 torr) was significantly higher than that during normoxia and those during hypoxia ($P < 0.01$). On the other hand, when Pa_{CO₂} was decreased from normocapnia to hypocapnia while maintaining Pa_{O₂} at a constant hypoxic level (Pa_{O₂} = 51.6 ± 1.7 torr), there was a significant increase in L_{R(plateau)} ($P < 0.01$).

It can be observed from figure 2 that the duration of increased glottic pressure, which was sustained for a period longer than the stimulus duration, was considerably longer during hypocapnia than during both normocapnia and hypercapnia. Figure 5 shows the effects of Pa_{CO₂} and Pa_{O₂} on the after-effect duration

(AED) which represents the period from the termination of SLN stimulation to the point where the elevated upper tracheal pressure returns to the prestimulation level. It can be seen that the relationships of AED to Pa_{CO₂} and Pa_{O₂} result in similar curves to those observed in figure 3 and figure 4 relating the L_{R(plateau)} to Pa_{CO₂} and Pa_{O₂}, respectively.

RELATIONSHIP BETWEEN VENTILATION AND LARYNGEAL RESISTANCE

As expected, ventilation increased as the chemical ventilatory drive was increased. Figure 6 shows an example of the relationship of L_{R(plateau)} to ventilation obtained from a cat breathing spontaneously under various conditions. All points fell along the same line regardless of the type and/or level of chemical drive. This linear relationship between L_{R(plateau)} and ventilation was observed in all of the animals.

Discussion

The method employed in this study allows quantitative measurements of laryngeal resistance to airflow and, therefore, degree of laryngospasm. We deliberately employed a slow rate of laryngeal airflow since a

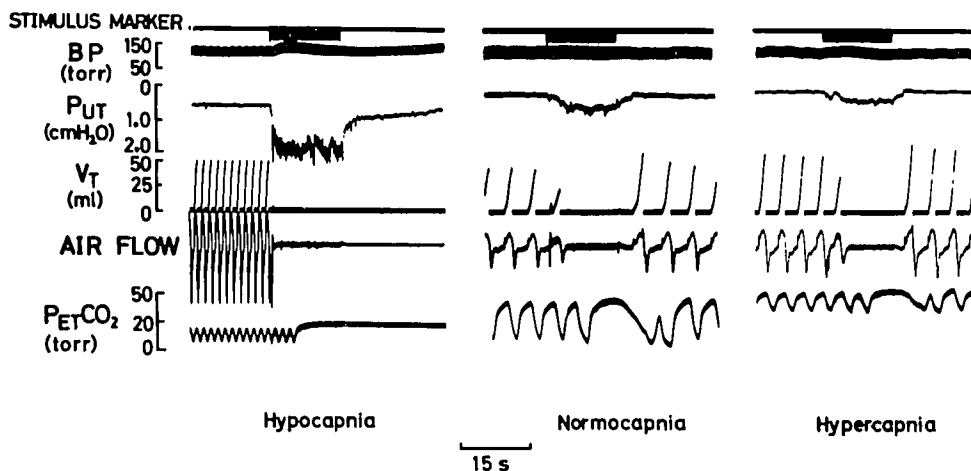


FIG. 2. Experimental records illustrating changes in the upper tracheal pressure (P_{UT}) and in respiration during SLN stimulation at three different levels of Pa_{CO₂} (hypocapnia, Pa_{CO₂} = 18.6 torr, Pa_{O₂} = 390 torr; normocapnia, Pa_{CO₂} = 33.2 torr, Pa_{O₂} = 385 torr; hypercapnia, Pa_{CO₂} = 48.5 torr, Pa_{O₂} = 380 torr). The duration of SLN stimulation was indicated by stimulus marker.

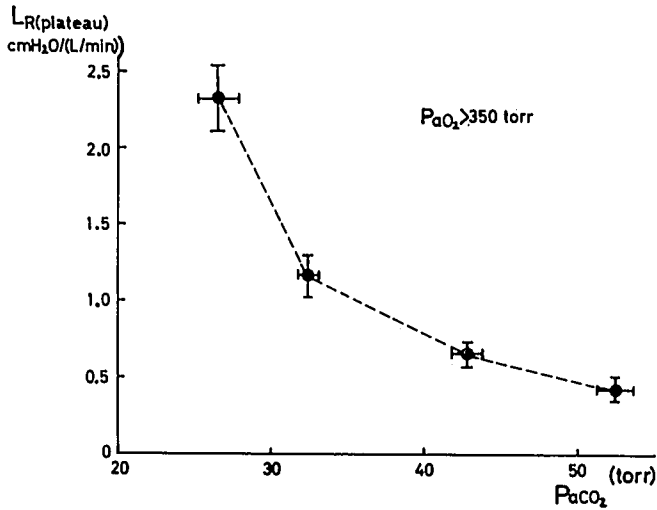


FIG. 3. The relationship between $[L_{R(\text{plateau})}]$ and $P_{a\text{CO}_2}$. As $P_{a\text{O}_2}$ increased, $L_{R(\text{plateau})}$ decreased progressively. The values represent means \pm SE ($n = 10$).

higher airflow would have produced undesirably high upper tracheal pressure, especially during laryngospasm. Such a slow rate of laryngeal airflow might cause little change in $L_{R(\text{baseline})}$. Our results, however, confirmed the observations of Campbell *et al.*¹² that hypercapnia and hypoxia lead to an increase in size and a decrease in resistance to flow in the laryngeal channel, whereas hypocapnia leads to a decrease in size and an increase in resistance to flow. Our finding that laryngospasm was attenuated as ventilation increased due to an increase in chemical ventilatory drive factors is in agreement with the results of Suzuki and Sasaki,⁴ but is in conflict with the observations of Wyke,¹³ who

noted that asphyxia precipitated spasm of the laryngeal adductor muscles.

Two possibilities might be considered with regard to the effects of chemical factors on the generation of laryngospasm. First, the finding that the $L_{R(\text{plateau})}$ decreased linearly as ventilation increased (fig. 6) suggests that there may be a central mechanism which determines the activity of the laryngeal reflex and maintains it in inverse proportion to the activity of the respiratory center which is in turn controlled by the level of chemical ventilatory drive. Suzuki and Sasaki⁴ studied the responses of the adductor motoneuron in the larynx to hyperventilation and hypoventilation during SLN stimulation in paralyzed cats. They observed that hyperventilation increased, whereas hypoventilation decreased, the activity of the adductor motoneuron and attributed these effects to preferential abolition of postsynaptic potentials by hypoxia due to hypoventilation. Although our observation that hypoxia decreased laryngeal responses to SLN stimulation is not incompatible with their results, our results also showed that hypocapnia facilitated, and hypercapnia attenuated the degree of laryngospasm in the absence of hypoxia. Moreover, SLN stimulation caused a strong laryngeal response during hypocapnic hypoxia. Thus, it is obvious that laryngeal responses to SLN stimulation are affected not only by the level of $P_{a\text{O}_2}$ but also by the level of $P_{a\text{CO}_2}$. In fact, it appears that CO₂ exerts the predominant influence over the production of laryngospasm.

There is much evidence in the literature that in the majority of neurons in the mammalian central nervous system, a rise in $P_{a\text{CO}_2}$ is accompanied by a hyper-

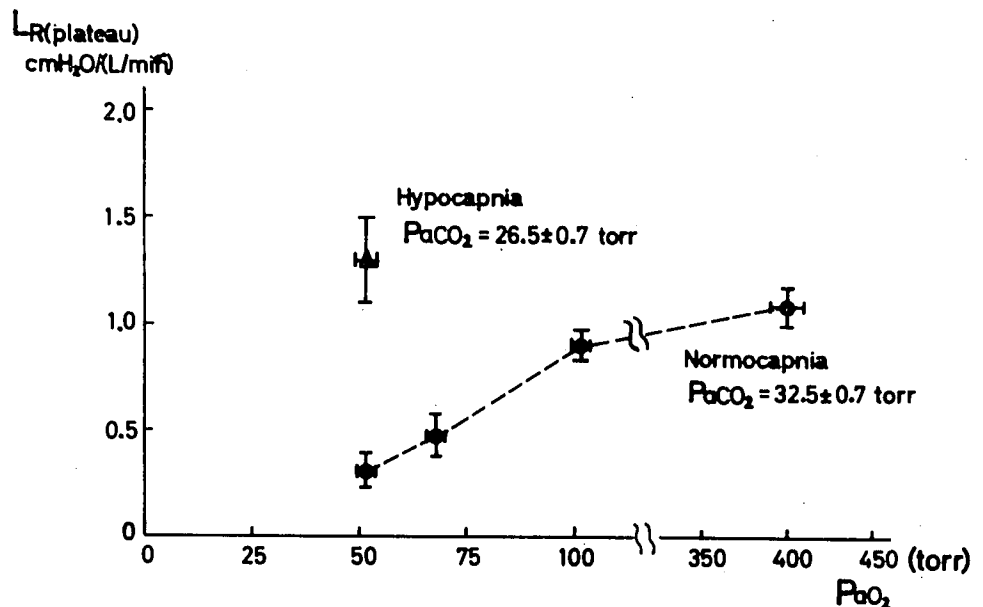


FIG. 4. The relationship between $L_{R(\text{plateau})}$ and $P_{a\text{O}_2}$. As $P_{a\text{O}_2}$ decreased at a constant normocapnic $P_{a\text{CO}_2}$, $L_{R(\text{plateau})}$ decreased progressively. A decrease in $P_{a\text{CO}_2}$ while maintaining $P_{a\text{O}_2}$ at a hypoxic level reverses this effect.

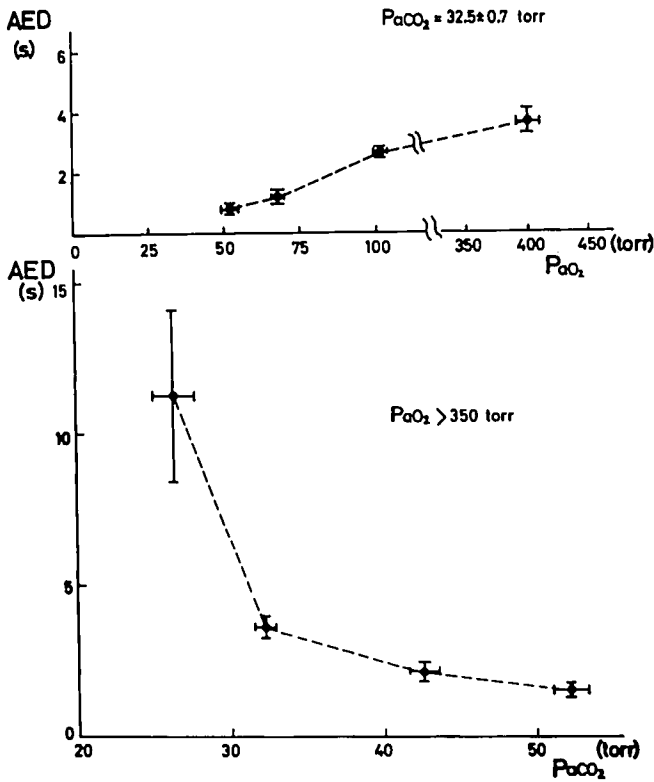


FIG. 5. The relationship between AED (after-effect duration) and changes in PaO₂ and PaCO₂. AED progressively shortens as the PaO₂ is decreased and as the PaCO₂ is increased, i.e., as the level of the chemical stimulation is increased. The shapes of these two curves are quite similar to those observed for PaCO₂ in figure 3 and for PaO₂ in figure 4.

polarization, indicating a reduction in the excitability of these neurons.¹⁴⁻¹⁶ For example, Mitchell and Herbert¹⁵ studied the effects of changes in PaCO₂ on the membrane potential of respiratory neurons in the nucleus ambiguus and the nucleus retroambiguus.

They found that the direct effect of increased PaCO₂ was to hyperpolarize the membrane potential of the respiratory neurons, indicating that increased PaCO₂ causes decreased excitability of respiratory neurons in the absence of input from the chemoreceptors. Decreased PaCO₂ caused the membrane potentials of these neurons to depolarize.¹⁵ Taking into consideration that neurons responsible for the laryngeal closure reflex are located in the same vicinity of the medulla oblongata¹⁷ as the respiratory neurons described above,¹⁵ it may be that the neurons responsible for the laryngeal closure reflex are also hyperpolarized in response to increases in PaCO₂. A second possibility with regard to the generation of laryngospasm relates to the possible effects of chemical factors on the peripheral neuromuscular system itself. Since the various muscles of the glottis are striated muscle,³ it is possible that changes in chemical factors affect neuromuscular transmission directly and thereby influence the degree of laryngospasm induced by SLN stimulation. Neuromuscular transmission has been shown to be facilitated during hypocapnia, whereas hypercapnia has the opposite effect.¹⁸ Also, it is possible that changes in chemical factors alter the active force of the muscles of the glottis by changing calcium conductance and/or sodium ion fluxes.¹⁹ Indeed, it has been shown that an increase in PaCO₂ and a decrease in the extracellular pH have a negative inotropic effect on isolated papillary muscles.²⁰

We showed in this study that the after-effect duration of laryngospasm was prolonged during hypocapnia but was shortened during hypercapnia and hypoxia. These observations, together with the findings of Suzuki and Sasaki⁴ that repetitive SLN stimulation produced numerous excitatory after-discharges in adductor motoneurons, suggest that it is more likely that facilitation of laryngospasm during

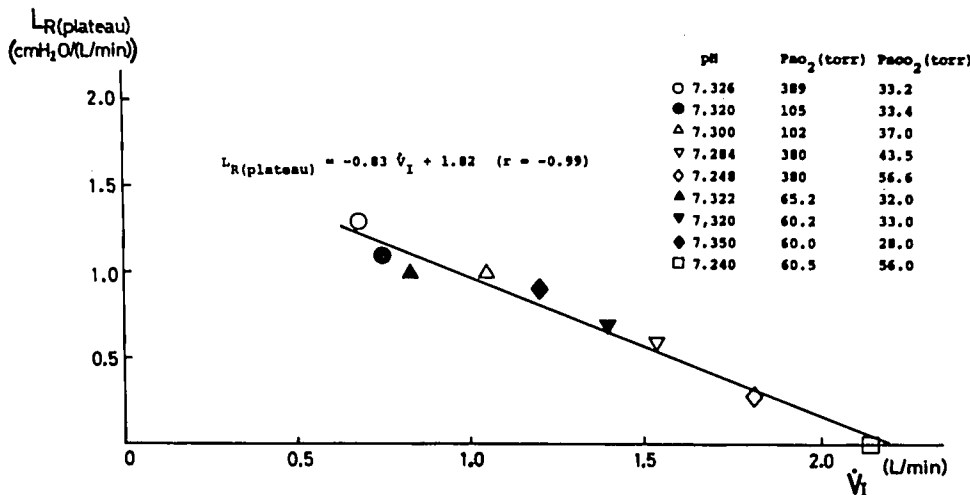


FIG. 6. The linear relationship between LR(plateau) and the inspired minute ventilation obtained from a cat breathing spontaneously under various levels of chemical ventilatory drive.

hypocapnia, and attenuation during hypoxia and hypercapnia are due to a central mechanism.

Although we demonstrated in this study that Pa_{O₂} and Pa_{CO₂} can exert considerable influence over laryngospasm in the cat, the simple extrapolation of our results to other species, particularly to humans, may not be entirely valid: for instance, we considered only the intrinsic laryngeal muscles in the occurrence of laryngospasm. However, there is some evidence that the extrinsic muscles are involved in various closing efforts of the larynx in humans.²¹

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