Responses to Severe Hypoxia of Phrenic and Recurrent Laryngeal Nerve Activity in Vagotomized Cats

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Nishino, T., Mizuguchi, T. and Honda, Y. Responses to Severe Hypoxia of Phrenic and Recurrent Laryngeal Nerve Activity in Vagotomized Cats. Tohoku J. Exp. Med., 1988, 156, Suppl., 57-64 — We investigated changes in activities of phrenic nerve (PN) and the recurrent laryngeal nerve (RLN) during progressive hypoxia produced by administration of a mixture of 5% O₂ in N₂ and a mixture of 5% O₂ in N₂O in 8 vagotomized, paralyzed, and artificially ventilated cats anesthetized with halothane. During progressive hypoxia produced by administration of 5% O₂ in N₂, both PN and RLN activities initially increased and then decreased at approximately the same rate. The relationship between PN and RLN activities during the respiratory stimulation and the relationship between PN and RLN activities during the depression due to hypoxia were both linear and were represented by the same linear regression line. The responses of PN and RLN activities to progressive hypoxia produced by administration of 5% O₂ in N₂O were basically similar to those observed during administration of 5% O₂ in N₂ although a concomitant increase in depth of anesthesia with N₂O enhanced the occurrence of hypoxic respiratory depression. These results suggest that the respiratory modulation of recurrent laryngeal motoneuron activity is closely related to that of phrenic motoneuron activity and that both motoneurons share similar control mechanisms. Neither severe hypoxia nor addition of N₂O to a halothane-anesthetized cat seems to affect the close linear relationship between PN and RLN activities.

It has been suggested that the respiratory control of the larynx plays a crucial role in maintaining upper airway patency (Bartlett et al. 1973; Orem and Lydic 1978; Orem et al. 1980). During mild-to-moderate levels of hypoxia, respiration progressively increases due to increasing activity of peripheral chemoreceptors. In contrast, severe hypoxia can cause respiratory depression, presumably due to a direct action of hypoxia on the respiratory center.

The study of Weiner et al. (1982) showed that during mild-to-moderate levels of hypoxia, increases in activity of the recurrent laryngeal nerve (RLN)
innervating the intrinsic laryngeal muscles are linearly related to increases in activity of the phrenic nerve (PN) innervating the diaphragm. Such a linear relationship between RLN and PN activities during hypoxic respiratory stimulation would help to prevent airway closure or promote maintenance of an open airway since it has been suggested that maintenance of upper airway patency depends on the balance between the activities of chest wall and upper airway muscles (Remmers et al. 1978). There is little information about the relationship between PN and RLN activities during severe hypoxia. Accordingly, in the present study we attempted to clarify the relationship between PN and RLN activities during progressive hypoxia continued to the point of apnea.

An additional question of interest is whether or not increasing depths of anesthesia combined with progressive hypoxia alter the relationship between PN and RLN activities during severe hypoxic respiratory depression. Therefore, in this study progressive hypoxia was produced by administration of the two different hypoxic gas mixtures: a mixture of 5% oxygen (O2) in nitrogen (N2) and a mixture of 5% O2 in nitrous oxide (N2O).

MATERIALS AND METHODS

Eight adult cats (2.5-3.8 kg) were anesthetized with halothane. The surgical procedure was similar to that described previously (Nishino et al. 1985), and will be described here only briefly. The animals were prepared with tracheal, arterial, and venous cannulae. The rectal temperature of the animals was maintained close to 38°C. End-tidal PCO2 (PETCO2) and end-tidal PO2 (PETO2) were continuously monitored with an infrared CO2 analyzer and a polarographic O2 analyzer, respectively. Both PN and RLN were isolated, desheathed, and cut in the right side of the neck. Their central ends were placed on bipolar silver electrodes in a pool of warm liquid paraffin and prepared for recording of their discharges. The activities of these nerves were amplified individually by a.c. amplifiers with 100-Hz and 3000-Hz low- and high-frequency filters. The rectified signals of the amplified signals were integrated by leaky R-C integrators having a time constant of 100 ms. Bilateral vagotomy was performed by cutting the right vagus below the origin of the RLN and the left vagus at the mid-cervical level. The animals were paralyzed with intravenous pancuronium bromide (0.3 mg/kg), and artificially ventilated with a gas mixture of 1.0% halothane in O2, adjusting volume and frequency to maintain PETCO2 at 35-40 mmHg. When an animal was in a steady-state condition, after obtaining 60 sec of control data (baseline) a hypoxic gas mixture of either 5% O2 in N2 or 5% O2 in N2O was delivered through a halothane vaporizer (Fluotec Mark 2, Cyparane, England) while the inspired concentration of 1.0% halothane was maintained. The order of the hypoxic gas mixture was randomized. Administration of the hypoxic gas mixture was continued until phasic PN activity disappeared. At this point, an arterial blood sample was taken and the hypoxic gas mixture was switched to 100% O2. The blood gas sample was analyzed immediately for pH, PCO2, and PO2 using an IL blood gas analysis system (IL system 1307, MA, USA). With each animal, following administration of one hypoxic gas mixture, sufficient time (5-10 min) was allowed before the administration of another hypoxic gas mixture to enable arterial blood pressure and PN and RLN activities to return to approximately the baseline level (90-100% of the baseline).

PN and RLN activities, their integrated activities, arterial blood pressure, PETCO2, and PETO2 were all recorded on ultra-violet-sensitive paper throughout the course of progressive hypoxia. The intensity of PN and RLN activities was quantified by the peak height of the
integrated activity. For the purpose of comparing various animals, percentage changes in nerve activities were calculated using a value of 100% for the maximum activity reached during administration of the hypoxic gas mixture of 5% O₂ in N₂. Statistical analysis was performed using paired t-test when appropriate.

**RESULTS**

**Time-course of changes in PN and RLN activities during progressive hypoxia**

Fig. 1A and 1B show the responses of PN and RLN activities in a representative animal to progressive hypoxia produced by administration of 5% O₂ in N₂ and 5% O₂ in N₂O, respectively. As hypoxia proceeded during administration of 5% O₂ in N₂ (Fig. 1A), PN and RLN activities progressively increased at approximately the same rate until PₑT₀₂ reached about 25 mmHg. A further decrease in PₑT₀₂ was associated with decreasing activities of PN and RLN. During the course of this respiratory depression, decreases in PN and RLN activities occurred in parallel fashion up to the point in time when PN activity nearly disappeared while the phasic RLN activity was replaced by a tonic RLN activity. The changes in PN and RLN activities during administration of 5% O₂ in N₂O were basically similar to those observed during administration of 5% O₂ in N₂ (Fig. 1B). However, compared with the responses during administration of 5% O₂ in N₂.

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**Fig. 1.** Changes in PN and RLN activities during progressive hypoxia produced by administration of 5% O₂ in N₂ (A) and 5% O₂ in N₂O (B).

BP = Arterial blood pressure; PNA = phrenic nerve activity; PLNA = recurrent laryngeal nerve activity; IPNA = integrated phrenic nerve activity; IRLNA = integrated recurrent laryngeal nerve activity; PₑT₀₂ = end-tidal PO₂; PₑTCO₂ = end-tidal PCO₂.
Table 1. Values of PO₂ at hypoxic respiratory depression (HRD) onset and hypoxic apnea (HA) reset during administration of 5% O₂ in N₂ and 5% O₂ in N₂O

<table>
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<th>Cat No.</th>
<th>P〈&lt;sub&gt;ET&lt;/sub&gt; O₂ at HRD onset (mmHg)</th>
<th>P〈&lt;sub&gt;A&lt;/sub&gt; O₂ at HA onset (mmHg)</th>
<th>P〈&lt;sub&gt;ET&lt;/sub&gt; O₂ at HRD onset (mmHg)</th>
<th>P〈&lt;sub&gt;A&lt;/sub&gt; O₂ at HA onset (mmHg)</th>
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Mean ± S.D. 27.6 ± 4.6 19.8 ± 4.3 32.2 ± 4.4** 26.8 ± 4.9**

**p < 0.01 (significantly different from corresponding values during administration of 5% O₂ in N₂, paired t-test).

N₂, the responses were much less and the respiratory depression occurred much earlier and at a higher level of P〈<sub>ET</sub> O₂. Table 1 summarizes the values of P〈<sub>ET</sub> O₂ and P〈<sub>A</sub> O₂ at which hypoxic respiratory depression and hypoxic apnea occurred, respectively, during administration of two different hypoxic gas mixtures. These data show that the values of P〈<sub>ET</sub> O₂ at onset of hypoxic respiratory depression and the values of P〈<sub>A</sub> O₂ at hypoxic apnea during administration of 5% O₂ in N₂O were significantly higher than those obtained during administration of 5% O₂ in N₂, indicating that a concomitant increase in depth of anesthesia with N₂O enhances the occurrence of hypoxic respiratory depression during progressive hypoxia.

Relationship of PN and RLN activities during progressive hypoxia

Fig. 2 shows the relationships between phasic PN and RLN activities during administration of 5% O₂ in N₂ obtained in all the animals. It can be seen that during progressive hypoxic stimulation, the relationships between PN and RLN activities are essentially linear in all the animals and that during hypoxic respiratory depression the relationships between PN and RLN activities are substantially identical to the relationships between PN and RLN activities obtained during the hypoxic stimulation. Fig. 3 shows the relationships between phasic PN and RLN activities during administration of 5% O₂ in N₂O. Although depression of hypoxic respiratory response by N₂O is evident, the relationships between PN and RLN activities during hypoxic depression are linear and basically similar to those observed in Fig. 2.
Fig. 2. Relationships between integrated PN and RLN activities during administration of 5% O₂ in N₂.
Circles and triangles indicate the relationships during hypoxic respiratory stimulation and hypoxic respiratory depression, respectively. Linear regression relationships between PN and RLN, calculated by the least squares method are also shown.

Fig. 3. Relationships of integrated PN and RLN activities during administration of 5% O₂ in N₂O. Symbols are the same as in Fig. 2.

**DISCUSSION**

There is much evidence (Miller and Tenney 1975; Weiskoph and Gabel 1975; Lahiri 1976) that the respiratory response to acute hypoxia is the net effect of a stimulating drive from the peripheral chemoreceptors and a depressant effect on the respiratory centers. The respiratory depression produced by hypoxia may not be recognizable at mild-to-moderate levels of hypoxia since the increasing periph-
eral chemoreceptor activity counteracts the depressant effect of hypoxia. Morril et al. (1975) reported that in dogs, ventilation increased until a $P_AO_2$ of about 20 mmHg was reached, and that further decreases in $P_AO_2$ were associated with decreasing ventilation. A similar observation was reported by Chernaick et al. (1970/71) who measured integrated phrenic nerve activity as an index of ventilatory effort in anesthetized and paralyzed dogs. In addition to these studies, Guntheroth and Kawabori (1975) showed that severe hypoxia produced initial hyperventilation and then hypoxic apnea at $PaO_2$ of about 8 mmHg. The responses of respiration to severe hypoxia observed in these studies are basically the same as those observed in our study. However, there is a slight difference between the results of these studies and ours in terms of severity of hypoxia which produced hypoxic respiratory depression. The values of $P_{ET}O_2$ at which the respiratory depression occurred and the values of $PaO_2$ at which apnea occurred are considerably higher in our study than those reported previously. The reasons for these differences are not clear, but they may relate to the fact that we used halothane as a background anesthetic whereas either chloralose or pentobarbital was used in other studies. Thus, although Morril et al. (1975) concluded that hypoxic ventilatory depression was not influenced by chloralose anesthesia, it is quite possible that the type of anesthetic agent and/or the level of anesthetic depth may influence the process of hypoxic respiratory depression. This possibility is supported by our finding that increasing depths of anesthesia with $N_2O$ further increase the values of $P_{ET}O_2$ and $PaO_2$ at which respiratory depression and apnea occur.

The present study confirms the observation of Weiner et al. (1982) in anesthetized and paralyzed dogs that mild-to-moderate levels of hypoxia cause PN and RLN activities to increase in parallel and proportionally. Furthermore, the present study demonstrates that during respiratory depression due to severe hypoxia, linear decreases in PN and RLN activities occur, and that the relationships between PN and RLN activities in these decreases are the same as the PN vs. RLN relations obtained during hypoxic respiratory stimulation. Although our results show that administration of 5% $O_2$ in $N_2O$ not only depresses the hypoxic stimulatory effects on respiration but also enhances the occurrence of hypoxic respiratory depression, the linear relationship between PN and RLN activities is maintained during respiratory depression. Similar linear decreases in PN and RLN activities have previously been shown to occur in response to baroreceptor stimulation (Salamone et al. 1983), dopamine administration (Van Lunteren et al. 1984), and increasing depths of anesthesia with halothane and enflurane (Nishino et al. 1985). All these observations suggest that the respiratory modulation of recurrent laryngeal motoneuron activity is closely related to that of phrenic motoneuron activity, and that conditions which cause changes in respiratory activity will little affect the close, linear relationship between PN and RLN activities.
In the present study we made no systematic examination of the tonic RLN activity which appeared during severe hypoxia. However, the effect on RLN activity of severe hypoxia is quite analogous to that of very deep anesthesia in that the tonic RLN activity appears after the cessation of the phasic inspiratory activity (Nishino et al. 1985). Since both severe hypoxia and deep anesthesia greatly depress the central nervous system, it seems that the appearance of the tonic RLN activity is associated with the depression of central nervous system functions. In this context, it is worthy to note that the tonic activity in the expiratory muscles can be induced by focal cooling of various medullary structures or focal microinjection of local anesthetic (Budzinska et al. 1985)

It has been suggested that maintenance of upper airway patency depends on the balance between the activities of chest wall and upper airway muscles, and that any conditions which preferentially decrease upper airway muscle activity would favor airway closure (Weiner et al. 1982; Van Lunteren et al. 1984). In this regard, there is much evidence (Salamone et al. 1983; Van Lunteren et al. 1984; Nishino et al. 1985) to suggest that during respiratory depression due to various respiratory depressants, hypoglossal nerve or genioglossal muscle activity more preferentially decreases than does PN or diaphragm activity, which might lead to oropharyngeal obstruction. The results of the present study indicates that during the respiratory depression produced by various respiratory depressants including hypoxia, laryngeal airway patency during spontaneous breathing would be well maintained due to close, linear relationship between phasic PN and RLN activities. In fact, airway obstruction at the larynx during respiratory depression has been reported infrequently.

References

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