Effects of Transient Hypoxia on Internal Intercostal Muscle Activity in Vagotomized Rabbits

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Abstract The effects of transient hypoxia on the responses of internal intercostal (IIC) muscle activity before and after surgical denervation of the carotid sinus nerves were studied in the bilaterally vagotomized rabbits. Bilateral vagotomy caused a complete inhibition of IIC activity in 11 of 13 animals. In the vagotomized animals with no expiratory activity in the IIC muscles, lung inflations with low O₂ gas mixture (6% O₂ in N₂) produced an increase in IIC activity, whereas these excitatory effects were remarkably reduced after sectioning the carotid sinus nerves. In addition, the effects of lung inflation with low O₂ gas mixture (6% O₂ in N₂) on carotid chemoreceptor activity were also studied in the vagotomized rabbits. Lung inflations with hypoxic gas caused an increase in carotid chemoreceptor activity. These results indicate that in the absence of proprioceptive input from the lungs, hypoxic stimulation of the carotid chemoreceptors is capable of activating the expiratory activity in the IIC muscles.

Key words: carotid sinus nerve, hypoxia, internal intercostal muscle activity, lung inflation, vagotomy.

The afferent activity in the vagus nerve is considered to be an important factor in determining the expiratory muscle response during quiet breathing, since bilateral vagotomy abolishes or greatly diminishes the electrical activity of expiratory muscles (Bishop, 1964; Kelsen et al., 1977; Arita and Bishop, 1983). The expiratory activity in the abdominal muscles is activated by applying continuous positive pressure breathing (CPPB) and in such a condition an increase in chemical stimuli has no effect on abdominal muscle activity (Bishop and Bachofen, 1972, 1973). Similar results were also obtained in the work of Kelsen et al. (1977), who demonstrated that the increase in abdominal muscle activity in response to hypoxia was seen during normal breathing, whereas the hypoxia-induced excitation in
abdominal muscle activity was remarkably suppressed when 8 cmH₂O continuous positive airway pressure (CPAP) was applied to the animal. These observations suggest that the increase in vagal afferent activity tends to inhibit the response of expiratory muscle activity to hypoxia.

On the other hand, a number of studies have shown that hypoxia elicits an increase in expiratory muscle activity (Gautier et al., 1973; Younes et al., 1974; Kelsen et al., 1977). These studies, however, did not examine the alteration of expiratory muscle activity after carotid chemodenervation. Recently, Matsumoto (1986) found that an intracarotid injection of sodium cyanide causes an increase in internal intercostal (IIC) muscle activity and the effect is completely eliminated by the section of the carotid sinus nerve. The results suggest that the excitatory response of IIC muscle activity to sodium cyanide injection is mainly mediated through the carotid chemoreflex. In the absence of any changes in proprioceptive inputs from the lungs and chest and abdominal walls, hypoxia still activates abdominal expiratory nerve activity (Ledlie et al., 1983). The question arises whether hypoxic stimulation of the carotid chemoreceptors has an excitatory action on expiratory muscle activity in the vagotomized animals.

By using the technique of lung inflation with hypoxic gas (6% O₂ in N₂), the present study investigated the effects of transient hypoxia on respiratory muscle activities (internal and external intercostal muscles and diaphragm) before and after surgical denervation of the carotid sinus nerves in the bilaterally vagotomized rabbits. In addition, the effects of lung inflation with hypoxic gas on carotid chemoreceptor activity were also studied in the bilaterally vagotomized rabbits.

MATERIALS AND METHODS

Experiments were performed on 16 rabbits weighing 2.5–3.5 kg. The animals were anesthetized with pentobarbital sodium (30 mg/kg) through the ear vein. The trachea was cannulated to obtain spontaneous breathing with room air. The femoral artery was cannulated for measurement of systemic blood pressure and for blood sampling. The femoral vein was also cannulated for injections of anesthetic drug and saline. The anesthesia was maintained by additional doses of pentobarbital sodium (3–4 mg/(kg·h)). Prior to the cannulations, heparin (500 unit/kg) was given intravenously. In order to avoid reflexes mediated by the aortic body, the aortic nerves were sectioned in advance. The rectal temperature was maintained at around 37°C by using a heating lamp. The muscle activity was recorded with two pairs of silver steel hooks. The electrodes were inserted into the sternal portion of the diaphragm through a small incision. The pectoralis and serratus anterior muscles on one side were detached to obtain access to the intercostal muscles (T₆–T₉). The external intercostal (EIC) muscles were partly detached to reach the IIC muscle portion. This procedure was done carefully to avoid pneumothorax and damaging the intercostal nerves. After setting the electrodes, the superficial muscles were sutured together. The muscle activity was amplified by a preamplifier, monitored on
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an oscilloscope, recorded on a polygraph and integrated with a 0.1 s time constant. After obtaining stable conditions, the vagus nerve was sectioned on both sides. After vagotomy, 11 of 13 animals showed no expiratory activity of the IIC muscles. Ten min after sectioning the vagus nerves, the stable conditions were determined by a measurement of both respiratory muscle activities and arterial blood gas tensions. The values of arterial $P_{O_2}$, $P_{CO_2}$, and pH were $86.3 \pm 2.6$ mmHg, $30.4 \pm 1.8$ mmHg, and $7.415 \pm 0.009$ (mean ± S.E., $n = 8$), respectively.

In the vagotomized animals with no expiratory activity of the IIC muscles, tidal volume ($V_T$) before lung inflation was measured by a pneumotachograph connected to a differential transducer. Lung inflations lasting from 10 to 15 s were performed at the end-expiratory phase (FRC) by using a syringe filled with hypoxic gas (6% $O_2$ in $N_2$). Hypoxic gas in a volume of 50 ml ranging from 1.2 to 1.6 $V_T$ was injected into the lungs immediately and the tubing (dead space, 3 ml) connected to the tracheal cannula was clamped. The carotid sinus nerves were identified and sectioned. The transection of the carotid sinus nerves was confirmed by the absence of characteristic inspiratory muscle responses following intravenous administration of sodium cyanide (60–100 µg) and/or inhalation of hypoxic gas (6% $O_2$ in $N_2$). Ten min after sectioning the carotid sinus nerves, blood was withdrawn from the femoral artery to measure $Pa_{O_2}$, $Pa_{CO_2}$, and pH and these values were $79.6 \pm 3.2$ mmHg, $35.8 \pm 2.1$ mmHg, and $7.392 \pm 0.009$ (mean ± S.E., $n = 8$), respectively. After obtaining stable conditions in respiratory muscle activities and arterial blood gas tensions, the same experimental procedures, as described previously, were repeated again. The amount of 50 ml used to inflate the lungs ranged from 1.7 to 2.3 times $V_T$ in the animals with sectioning of the carotid sinus nerves.

In another series of experiments used for three vagotomized rabbits, the left carotid sinus nerve was cut at its junction with the glossopharyngeal nerve. A few fibers were separated, placed on bipolar silver electrodes and submerged under warm liquid paraffin (37–38°C). The action potentials from the fibers were recorded. The carotid chemoreceptor afferents were initially identified by their sporadic firing pattern. The identification of the afferents was further confirmed by the following tests: decreased and increased activities by hyperoxia and hypoxia or hypercapnia, respectively. After obtaining stable conditions in the carotid chemoreceptor activity, lung inflations with hypoxic gas, as described previously, were performed.

RESULTS

Effects of sectioning the vagus nerves on respiratory muscle activities

Figure 1 shows typical responses of internal intercostal and external intercostal activities and diaphragm activity (DIAP) to bilateral vagotomy. The section of the vagus nerves on both sides elicited an immediate effect on respiratory muscle activities. It increased the peak amplitude of both integrated EIC activity and DIAP and the duration of inspiration, but decreased the rate of rise of both integrated EIC

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activity and DIAP. On the other hand, bilateral vagotomy inhibited the expiratory activity in the IIC muscles. Three or 4 min after sectioning the vagus nerves, a complete inhibition of the expiratory activity in the IIC muscles was observed in 11 or 13 animals. In the remaining 2 animals, the IIC activity was markedly suppressed after bilateral vagotomy.

**Effects of sectioning the carotid sinus nerves on the response of respiratory muscle activities to lung inflation with hypoxic gas in vagotomized rabbits**

The responses of simultaneous IIC, EIC, and DIAP recordings to lung inflation with hypoxic gas (6% O_2 in N_2) before and after sectioning the carotid sinus nerves are shown in Fig. 2.

A tonic activity in both IIC and EIC muscles appeared following lung inflation with low O_2 gas mixture. These tonic activities fired throughout the whole respiratory cycle and showed a low amplitude (Fig. 2A). An increase in both tonic IIC and EIC activities during lung inflation with hypoxic gas was greatly reduced after bilateral section of the carotid sinus nerve (Fig. 2B). The changes of tonic IIC and EIC activities in response to lung inflation with hypoxic gas in 11 vagotomized animals before and after sectioning the carotid sinus nerves are summarized in

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Table 1. Before the sectioning, the mean values of tonic JIC and EIC activities were 7.2 and 118,\(\mu V\), respectively. After the sectioning, both values decreased significantly to 2.3 and 45,\(\mu V\), respectively.

During lung inflation with hypoxic gas, the phasic activity of JIC and EIC muscles and the DIAP were remarkably augmented. These excitatory responses fell gradually after release of lung inflation (Fig. 2A). After sectioning the carotid sinus nerves, in this preparation there were no significant changes in JIC and EIC activities. A small and transient increase in JIC activity was only observed in the latter portion of lung inflation with low \(O_2\) gas mixture and this small effect declined rapidly after cessation of lung inflation (Fig. 2B). The results in 11 vagotomized animals are summarized in Table 2. The mean values of phasic EIC activity and

Table 1. The changes of tonic IIC and EIC activities in response to lung inflation with hypoxic gas before and after sectioning the carotid sinus nerve.

<table>
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<tr>
<th></th>
<th>Before sectioning the carotid sinus nerves</th>
<th>After sectioning the carotid sinus nerves</th>
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<tbody>
<tr>
<td>Tonic IIC activity ((\mu V))</td>
<td>7.2(\pm)0.3</td>
<td>2.3(\pm)0.2*</td>
</tr>
<tr>
<td>Tonic EIC activity ((\mu V))</td>
<td>118(\pm)11</td>
<td>45(\pm)5*</td>
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</table>

Mean \(\pm\) S.E., \(n=11\). IIC activity: internal intercostal activity, and EIC activity: external intercostal activity. * Statistical difference between the values before and after sectioning the carotid sinus nerves (\(p<0.05\)).
DIAP during quiet breathing, which were 236 and 242 µV, respectively, reduced to 214 and 221 µV after sectioning the carotid sinus nerves, but these values did not show any significant changes when compared to the control values. The increased values of phasic IIC and EIC activities and DIAP during lung inflation with hypoxic gas, which were 81, 817, and 857 µV, respectively, reduced significantly to 27, 378, and 344 µV after bilateral carotid sinus denervation.

The changes of mean systemic blood pressure (SBP) induced by lung inflation with hypoxic gas in 11 vagotomized animals before and after the section of the carotid sinus nerves are summarized in Table 3. The mean SBP which was between 114 and 134 mmHg during quiet breathing decreased to 118 mmHg during lung inflation with hypoxic gas. After sectioning the carotid sinus nerves, the mean SBP was 133 mmHg on the average during quiet breathing. The value of mean SBP in the

Table 2. The changes of phasic IIC and EIC activities and DIAP in response to lung inflation with hypoxic gas before and after sectioning the carotid sinus nerves.

<table>
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<th>Before sectioning the carotid sinus nerves</th>
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<tr>
<td></td>
<td>During air breathing</td>
<td>During lung inflation with hypoxic gas</td>
</tr>
<tr>
<td>Phasic IIC activity (µV)</td>
<td>0</td>
<td>81 ± 4</td>
</tr>
<tr>
<td>Phasic EIC activity (µV)</td>
<td>236 ± 19</td>
<td>817 ± 42*</td>
</tr>
<tr>
<td>DIAP (µV)</td>
<td>242 ± 21</td>
<td>857 ± 47*</td>
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Mean ± S.E., n = 11. IIC activity: internal intercostal activity, EIC activity: external intercostal activity, and DIAP: diaphragm activity. * Statistical difference between the values before and after sectioning the carotid sinus nerves (p < 0.05).

Table 3. The changes of mean systemic blood pressure in response to lung inflation with hypoxic gas before and after sectioning the carotid sinus nerves.

<table>
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<th>Before sectioning the carotid sinus nerves</th>
<th>After sectioning the carotid sinus nerves</th>
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<tbody>
<tr>
<td></td>
<td>During air breathing</td>
<td>During lung inflation with hypoxic gas</td>
</tr>
<tr>
<td>Mean SBP (mmHg)</td>
<td>124 ± 3</td>
<td>118 ± 4</td>
</tr>
</tbody>
</table>

Mean ± S.E., n = 11. SBP: systemic blood pressure.
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Animals with bilateral carotid sinus denervation decreased to 126 mmHg on the average during lung inflation with hypoxic gas. It is noted that the effects of lung inflation with hypoxic gas on the values of mean SBP did not show any significant changes when compared to the control values before and after sectioning the carotid sinus nerves.

Effects of lung inflation with hypoxic gas on carotid chemoreceptor activity in vagotomized rabbits

Figure 3 shows the typical response of carotid chemoreceptor activity to lung inflation with hypoxic gas in a bilaterally vagotomized animal. Lung inflation with low O₂ gas mixture caused an increase in carotic chemoreceptor activity. Similar effects were obtained in all of the five different carotid chemoreceptor fibers. The results are summarized in Table 4. The mean firing rate of carotid chemoreceptor activity during air breathing was 1.2 imp/s, and the number of spikes during lung inflation with hypoxic gas increased to 3.1 imp/s.

DISCUSSION

Sectioning of the vagus nerves caused a complete inhibition of IIC activity in 11 of 13 rabbits. In the remaining 2 rabbits, IIC activity was remarkably suppressed after bilateral vagotomy. Similar results in the cat's IIC activity suggest that the expiratory activity in the IIC muscles during quiet breathing may be dependent on the afferent activity from the pulmonary vagal receptors (ARITA and BISHOP, 1983). However, it is still debatable whether the depressing effect of vagotomy on expiratory muscle activity is mediated by the afferent input from slowly adapting
pulmonary stretch receptors or rapidly adapting pulmonary stretch receptors or some tonically active pulmonary vagal receptors being different from the pulmonary stretch receptors (Fishman et al., 1973; Davies et al., 1980; Farber, 1982; Arita and Bishop, 1983). Further studies would be needed to elucidate this problem.

During lung inflation with hypoxic gas, the IIC and EIC recordings were functionally divided into two different types: (1) the tonic activity with small amplitude fired throughout the whole respiratory cycle and (2) the phasic activity with large amplitude fired during expiration or inspiration. The responses of tonic activity in the IIC and EIC muscles to lung inflation with hypoxic gas were qualitatively similar to those of the phasic activity. The difference between the respiratory muscle activities with small amplitude and large amplitude would be determined by the cell size of respiratory motoneurons in the spinal cord (Henneman et al., 1965). Presumably, the tonic activity of respiratory muscles is innervated by the small respiratory motoneurons whereas the phasic activity of respiratory muscles is innervated by the large respiratory motoneurons.

In the vagotomized animals with no expiratory activity in the IIC muscles, lung inflations with hypoxic gas caused an increase in IIC activity and these effects were greatly reduced after sectioning of the carotid sinus nerves. In the absence of respiratory movements, Ledlie et al. (1983) have found that hypoxia increases the abdominal expiratory nerve activity. This increase would be mediated through the change in the carotid chemoreceptor activity and/or a direct action of hypoxia on the central and spinal neurons. In this study, the discharge rate of carotid chemoreceptor fibers in bilaterally vagotomized animals was increased during lung inflation with hypoxic gas. Recently, Lipski et al. (1984) have shown that the afferent input from the carotid chemoreceptors directly projects onto medullary expiratory neurons. Thus, excitation of the expiratory neurons produced by lung inflation with hypoxic gas would reflect the change of carotid chemoreceptor firing in response to hypoxia and which in turn causes an increase in IIC activity. This idea was further confirmed by the finding that the excitatory response of IIC activity to intracarotid administration of sodium cyanide was greater than that of EIC activity or DIAP (Matsumoto, 1986). The hyperpolarization of the membrane potential in spinal expiratory motoneurons during inspiration occurs as a result of the inhibitory postsynaptic potential (IPSP) generated by bulbospinal inspiratory neurons (Sears, 1977). Conversely, the depolarization of spinal expiratory motoneurons during expiration results from the excitatory postsynaptic potential (EPSP) generated by bulbospinal expiratory neurons (Kirkwood and Sears, 1973). These observations lead to the suggestion that the activity of the IIC muscles is strongly affected by altering the membrane potential of expiratory motoneurons during inspiration and expiration. Sears et al. (1982) have found that hypoxic stimulation of the peripheral chemoreceptors in artificially ventilated cats elicits a marked increase in inspiratory motoneuron activity with a reciprocal reduction of the expiratory motoneuron activity, suggesting that the increased IPSP of expi-
ratory motoneuron during inspiration may exert a predominant effect in determin-
ing the response of internal intercostal motoneuron activity to hypoxia. On the
other hand, there is evidence that the carotid chemoreceptor afferents directly
project to the medullary expiratory neurons (Lipski et al., 1976, 1984). In
spontaneously breathing rabbits, hypoxic stimulation of the carotid chemoreceptors
did not cause the inspiratory shift suggested by Sears et al. (1982) and the excitatory
responses of IIC and EIC activities and DIAP following lung inflation with hypoxic
gas were greatly reduced or were eliminated when the carotid sinus nerves were
sectioned. The observed discrepancies can possibly be attributed to the following:
the magnitude of increased EPSP in expiratory motoneurons due to transient
hypoxia is much greater than that of increased IPSP in this study. However, it
cannot rule out the possibility that the increase in bulbospinal inspiratory neuron
activity during transient hypoxia is not attained at a level to elicit a reciprocal
inhibition to the spinal expiratory motoneurons. Further study is needed to
elucidate this mechanism.

Arita and Bishop (1983) reported that lung inflation after vagotomy still
activated the expiratory activity in the IIC muscles. This excitation would be
explained by the result of activation of the segmental inputs from proprioceptive
receptors of intercostal muscles (Eklund et al., 1964; Sears, 1964). The fact that
lung inflation with hypoxic gas caused augmentation of the expiratory activity in the
IIC muscles in the vagotomized state suggests that hypoxic stimulation of the
carotid chemoreceptors would cause an increase in the proprioceptive receptor
activity of the intercostal muscles via a γ-loop system. After sectioning the carotid
sinus nerves, the excitation of IIC activity to the same lung inflation was small and
only appeared in the latter period of lung inflation. This effect was not similar to the
findings of Arita and Bishop (1983), who demonstrated that lung inflation (room
air, 100 ml above FRC) ranging from 3 to 4 VT produced an immediate effect on IIC
activity in vagotomized cats. In vagotomized rabbits with sectioning of the carotid
sinus nerves, lung inflation (50 ml above FRC) ranging from 1.7 to 2.3 times VT did
not cause an immediate effect on IIC activity. The difference between their results
and the present study would have been resulted from the difference in inflation
volume. A small effect of IIC activity during lung inflation with hypoxic gas in the
vagotomized rabbit with carotid sinus denervation would occur as the result of
activation of the spinal reflex being independent on the afferent input from carotid
chemoreceptors.

The decreased activity of the carotid baroreceptors resulting from hypotension
activates abdominal muscle activity (Bishop, 1974). In this study, the values of mean
SBP, when lung inflations using hypoxic gas were applied to the vagotomized
animals with and without intact carotid sinus nerves, did not show any statistical
difference in comparison with those before lung inflation. Thus, it is most likely that
the increase in IIC activity during lung inflation with low O2 gas mixture is not
mediated by the change in carotid baroreceptor activity.
REFERENCES


