Chronic Intermittent Asphyxia Impairs Rat Upper Airway Muscle Responses to Acute Hypoxia and Asphyxia*

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**Background:** Obstructive sleep apnea (OSA) is a major clinical disorder that is characterized by multiple episodes of upper airway obstruction due to the failure of the upper airway dilator muscles to maintain upper airway patency. This results in chronic intermittent asphyxia (CIA) due to repetitive apneas, but very little is known about the effects of CIA on upper airway muscle function.

**Objective:** To test the hypothesis that CIA affects upper airway muscle activity and electromyogram (EMG) responses to acute hypoxia and asphyxia.

**Design:** Record upper airway EMG responses to acute hypoxia and asphyxia in control and CIA-treated rats.

**Setting:** Department of Physiology, Royal College of Surgeons in Ireland, Dublin, Ireland.

**Measurements:** Sternohyoid (SH) muscle and diaphragm (DIA) muscle EMG activities were recorded in both groups during normoxia, hypoxia (7.5% O₂ in N₂), and asphyxia (7.5% O₂ and 3% CO₂) under pentobarbitone anesthesia.

**Results:** Baseline SH EMG activity was significantly elevated in the CIA-treated rats compared to the controls, whereas DIA EMG activity was similar in the two groups. In addition, CIA significantly reduced SH EMG but not DIA EMG responses to acute hypoxia and asphyxia.

**Conclusions:** The elevated upper airway muscle activity associated with OSA in humans during wakefulness is due at least in part to CIA. We propose that a reduction in the response of upper airway dilator muscles to acute asphyxia following upper airway obstruction is likely to cause further asphyxic insult, leading to a vicious feed-forward cycle exacerbating the condition. Our results suggest that CIA contributes to the pathophysiology of sleep-disordered breathing.

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**Key words:** diaphragm; electromyography; hypercapnia; hypoxia; long-term facilitation; obstructive sleep apnea; sleep-disordered breathing; sternohyoid; upper airway patency

**Abbreviations:** ANOVA = analysis of variance; CIA = chronic intermittent asphyxia; DIA = diaphragm; EMG = electromyogram; LTF = long-term facilitation; OSA = obstructive sleep apnea; SH = sternohyoid

Obstructive sleep apnea (OSA) has an incidence estimated at > 1% of the adult population.1,2 During sleep in OSA patients, there are multiple episodes of occlusion of the pharynx during inspi-
Thus, sleep apnea syndromes are characterized by chronic intermittent asphyxia (CIA) due to repetitive apneas, but little is known about the effects of CIA on upper airway muscle function.

We have developed a rat model that mimics the repetitive episodic asphyxia associated with OSA in humans. Exposing rats to alternating episodes of normoxia and asphyxia twice per minute for 8 h per day for 5 weeks increases hematocrit, causes right ventricular hypertrophy, and increases pulmonary arterial pressure. In the present study, we tested the hypothesis that chronic exposure to an episodic asphyxic stimulus adversely affects upper airway muscle responses to acute physiologic stimuli.

**Materials and Methods**

All procedures were performed in accordance with national legislation under the Cruelty to Animals Act 1876 and European Union Directive 86/609/EC. Experiments were carried out on 12 male Wistar rats that were randomly assigned to groups of 6 rats each. Except during the treatment and experimental periods, the rats were housed three to a cage under a 12 h/12 h (light/dark) photoperiod and were given free access to food and water.

Animals were weighed each day and were placed in restrainers with their heads surrounded by hoods. Using timed solenoid valves, as previously described, a mixture of N₂ and CO₂ was distributed into the hoods for 15 s at a flow rate that reduced the ambient O₂ concentration to 6 to 8% and increased the ambient CO₂ concentration to 12 to 14%. This was followed by an infusion of air for 15 s and the recovery of O₂ and CO₂ concentrations to room air values. This cycle was repeated twice per minute, 8 h per day, 5 days per week for 5 weeks. For the control group, animals were placed in identical restrainers and hoods, and received air that was switched to air from a separate source every 15 s using identical solenoid valves with the same flow rates as used in the CIA-treated group. This cycle was repeated twice per minute, 8 h per day, 5 days per week for 5 weeks.

The minimum O₂ concentration and maximum CO₂ concentration were measured three times daily by placing the probes of an O₂ analyzer (Engstrom Eliza Duo; Gambo-Engstrom AB; Bromma, Sweden) and a CO₂ analyzer (Capstar-100 end-tidal CO₂ analyzer; CWE Inc; Ardmore, PA) as close as possible to the nose of the rats for 5 min at approximately 0, 4, and 8 h of the treatment period. Mean daily values were calculated.

The treatment protocols were carried out during the daytime (8:00 AM to 4:00 PM). For both the control and CIA groups, blood samples were obtained from the tail in all rats to determine hematologic data (i.e., hemoglobin concentration and RBC count) at days 0, 7, 14, 21, 28, and 35 of the treatment period.

After the 5-week treatment period, animals were anesthetized (pentobarbitone sodium, 60 mg/kg intraperitoneally) and were placed in the supine position on a thermostatically controlled heating blanket. The core temperature was maintained at 37°C.

The signals were stored for later analysis on a computer. All signals were passed through an analog-to-digital converter and were recorded online using a commercial data acquisition system.

**Protocol**

EMG activities were measured while the rats were breathing from a bias flow of 2 L/min of air (baseline) that was switched to a mixture containing 7.5% O₂ in N₂ (hypoxia) or to a mixture containing 7.5% O₂ and 3% CO₂ (asphyxia) for 3 min. The order of the hypoxia and asphyxia trials was randomized, and sufficient time was allowed between trials (i.e., ≥5 min) to re-establish baseline conditions.

**Data and Statistical Analysis**

Phasic inspiratory SH and DIA EMG activities were quantified as the peak height of the integrated EMG signal from the end-expiratory level in arbitrary units. EMG burst frequency and peak amplitude were averaged for each min before and during the hypoxic and asphyxic trials. EMG measurements were normalized by expressing the peak height of the integrated EMG signal as a percentage of the average peak amplitude obtained during 9% CO₂ exposure (percentage of reference value). The 9% CO₂ trial (hypercapnia) was carried out at the end of the experiment after the hypoxia and asphyxia trials. All data were initially averaged for each animal.

Values are expressed as the mean ± SEM. The data were analyzed for statistical significance between the control and CIA groups using analysis of variance (ANOVA) and the Fischer least significant difference test, with p < 0.05 taken as the level of significance.

**Results**

There was no significant difference in body weight for the two groups for the duration of the treatment period (Table 1). At the end of the treatment period, under pentobarbitone anesthesia, phasic inspiratory SH EMG activity was observed in all animals.

**Effect of CIA on Hematologic Data**

At the beginning of the treatment period there was no significant difference between the two groups in hemoglobin concentration and RBC count (Table 1). However, both were significantly elevated in the CIA-treated rats on days 7, 14, 21, 28, and 35 of the treatment period (Table 1).

**Effect of CIA on Baseline EMG Activities**

Baseline phasic inspiratory SH EMG activity was significantly higher in CIA-treated rats compared to controls, with no overlap between the two groups (Fig 1). Baseline DIA EMG activity for CIA-treated rats was significantly lower than that for the control group prior to the hypoxic trials (Fig 2) but was not
significantly different from control values prior to the asphyxic trials (Fig 3). When baseline data for DIA EMG activity prior to all trials was pooled, there was no significant difference between control and CIA-treated rats (Fig 1). Baseline respiratory burst frequency was not significantly different between the two groups.

Effect of CIA on EMG Responses to Acute Hypoxia

SH and DIA EMG responses to hypoxia are summarized in Figure 2. The magnitude of SH EMG responses to hypoxia was significantly lower in CIA-treated rats compared to controls. In contrast, the magnitude of DIA EMG responses to hypoxia was not significantly different between the two groups (Fig 2). The respiratory frequency response to hypoxia was not significantly different in control and CIA-treated animals.

Effect of CIA on EMG Responses to Acute Asphyxia

SH and DIA EMG responses to asphyxia are summarized in Figure 3. The magnitude of SH EMG responses to asphyxia was significantly lower in CIA-treated rats compared to controls. With the exception of the early response to hypoxia (ie, the 1-min time point), the magnitude of DIA EMG responses to asphyxia was not significantly different between the two groups (Fig 3). The respiratory frequency response to asphyxia was not significantly different in control and CIA-treated rats.

Table 1—Body Weight and Hematologic Data for Days 0, 7, 14, 21, 28, and 35 of the Treatment Protocol in Control and CIA-Treated Rats*

<table>
<thead>
<tr>
<th>Data</th>
<th>Body Weight, g</th>
<th>Hemoglobin Concentration, g/dL</th>
<th>RBC Count, ×10⁶ cells/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>359 ± 3</td>
<td>14.46 ± 0.03</td>
<td>8.45 ± 0.09</td>
</tr>
<tr>
<td>Day 7</td>
<td>351 ± 3</td>
<td>14.41 ± 0.05</td>
<td>8.13 ± 0.14</td>
</tr>
<tr>
<td>Day 14</td>
<td>350 ± 4</td>
<td>13.99 ± 0.14</td>
<td>8.06 ± 0.05</td>
</tr>
<tr>
<td>Day 21</td>
<td>347 ± 5</td>
<td>13.89 ± 0.10</td>
<td>7.90 ± 0.05</td>
</tr>
<tr>
<td>Day 28</td>
<td>356 ± 3</td>
<td>14.43 ± 0.04</td>
<td>8.11 ± 0.01</td>
</tr>
<tr>
<td>Day 35</td>
<td>364 ± 3</td>
<td>14.34 ± 0.03</td>
<td>7.80 ± 0.03</td>
</tr>
<tr>
<td>CIA treated (n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>347 ± 3</td>
<td>14.20 ± 0.07</td>
<td>8.67 ± 0.04</td>
</tr>
<tr>
<td>Day 7</td>
<td>338 ± 3</td>
<td>16.00 ± 0.16†</td>
<td>9.99 ± 0.16†</td>
</tr>
<tr>
<td>Day 14</td>
<td>333 ± 3</td>
<td>16.53 ± 0.07†</td>
<td>10.81 ± 0.09†</td>
</tr>
<tr>
<td>Day 21</td>
<td>329 ± 3</td>
<td>16.07 ± 0.09†</td>
<td>11.07 ± 0.11†</td>
</tr>
<tr>
<td>Day 28</td>
<td>339 ± 4</td>
<td>16.45 ± 0.09†</td>
<td>10.28 ± 0.07†</td>
</tr>
<tr>
<td>Day 35</td>
<td>340 ± 4</td>
<td>16.00 ± 0.06†</td>
<td>10.01 ± 0.03†</td>
</tr>
</tbody>
</table>

*Values given as mean ± SEM.
†Significant difference from day 0 (CIA-treated), p < 0.05 (ANOVA); and significant difference from corresponding value (controls), p < 0.05 (ANOVA).

Discussion

We have developed a rat model that mimics the intermittent blood gas changes that are associated with sleep-disordered breathing in humans. This model can be viewed as having the advantage that it allows the study of the effects of long-term blood gas changes independent of the mechanical changes that occur during obstructed breaths in OSA patients, most notably the marked subatmospheric intrathoracic pressures that are generated. Exposing rats to episodic periods of normoxia and asphyxia twice per minute, 8 h per day, 5 days per week for 5 weeks causes pulmonary and systemic arterial hypertension, increased hematocrit, and right ventricular hypertrophy.

In the present study, we showed that CIA significantly increases baseline upper airway dilator EMG activity and impairs upper airway EMG responses to acute hypoxia and asphyxia. The elevated SH EMG activity during baseline (normoxia) conditions in
CIA-treated animals compared to controls is entirely consistent with reports of elevated upper airway muscle activity during wakefulness in patients with OSA compared with healthy subjects.9–11 It has been suggested that this represents a neuromuscular compensatory mechanism that is necessary to overcome anatomic limitations in OSA patients, thereby maintaining adequate airway patency during wakefulness.10,11 It is speculated that the loss of such compensatory mechanisms with the onset of sleep and the consequent decrease in upper airway muscle activity leads to airway collapse in patients who are at risk due to inadequate airway anatomy.10,11

Mezzanotte et al10,11 have suggested that the enhanced upper airway muscle activity in OSA patients during wakefulness is a reflex response that is thought to be driven, at least in part, by the greater intraluminal negative pressure that is generated during inspiration in OSA patients to overcome increased airway resistance. This hypothesis is further strengthened by observations that continuous positive airway pressure reduces upper airway muscle activity in OSA patients, it does not completely return phasic genioglossus activity to the levels seen in control individuals.10 These observations have led to suggestions that there may be additional contributing factors resulting in enhanced upper airway muscle activity during wakefulness in OSA patients.

In the present study, SH EMG activity was consistently elevated in CIA-treated animals compared to controls (48.5 ± 0.5% of reference values; reference value range, 44.0 to 51.8% vs 28.4 ± 0.3%; range, 26.9 to 30.5%). This does not simply reflect a global increase in respiratory motor output in CIA-treated animals (caused, for example, by an artificial increase in chemical drive under anesthesia), as
DIA EMG activity was not different between the two groups. Thus, we suggest that the elevated SH EMG activity seen under baseline conditions is caused by chronic exposure to episodic asphyxia. We propose that CIA is at least in part responsible for the elevated upper airway dilator muscle activity seen in OSA patients during wakefulness.9–11

The mechanisms responsible for the selective increase in upper airway muscle activity in CIA-treated rats remains unclear. It is tempting to speculate, however, that it represents persistent facilitation of cranial motor output to the upper airway muscles elicited by episodic asphyxia. Such long-term facilitation (LTF) of respiratory motor output has been well-described. Fuller et al14 and Mitchell et al15 have unequivocally demonstrated that intermittent hypoxia produces a long-lasting, serotonin-dependent facilitation of respiratory motor output. LTF is elicited by intermittent, but not by continuous, hypoxia in rats.14,15 demonstrating that the underlying mechanism is remarkably patternsensitive, which is consistent with other models of neuroplasticity. LTF requires serotonin receptor activation during, but not following, episodic hypoxia, indicating that serotonin is necessary to evoke, but not to maintain, LTF.16 Interestingly, LTF, which is an example of plasticity in respiratory motor control, is itself subject to considerable modification and can be altered by prior experience or conditioning (ie, metaplasticity of LTF).14,15 For example, LTF of phrenic motor output is enhanced following chronic intermittent hypoxia.14 Taken together, we tentatively suggest that the elevated SH EMG activity seen under baseline conditions in CIA-treated animals represents a form of LTF that is preferential to hypoglossal (cranial) vs phrenic (spinal) motor output. The expression of LTF is heterogenous among respiratory motor pools,17,18 and previous studies have demonstrated that the magnitude of LTF evoked in intercostal19 and hypoglossal14 motor outputs can exceed phrenic LTF.

However, there are a number of limitations in our study that must be considered. Our rat model of OSA7,8 assumes no difference in the structural properties of the upper airway between control and CIA-treated rats. We cannot discount the possibility,
however, that there are mechanical consequences of CIA due to, for example, increased ventilatory drive and/or the occurrence of augmented breaths during episodic asphyxia. CIA could be associated with mechanical changes in the upper airway that result in altered upper airway reflex function. For example, CIA is associated with altered autonomic function\(^{20}\) that could potentially influence upper airway resistance through effects on mucosal blood flow.\(^{21–25}\) There is also the potential for direct effects of intermittent asphyxia on the mucosal vasculature. Tahawi and coworkers\(^{26}\) have recently demonstrated altered vascular reactivity following chronic intermittent hypoxia in rats. Whatever the mechanism, CIA-induced narrowing of the upper airway would reflexively modulate cranial motor output to the upper airway dilator muscles (owing to the greater negative pressure generated during inspiration to overcome putative increases in upper airway resistance). Although we believe that such a mechanism is unlikely to be responsible for the elevated baseline SH EMG activity in CIA-treated rats, such a finding would be consistent with the neuromuscular compensatory mechanism described elsewhere.\(^{10,11}\)

Our bias, however, is to suggest that the elevated SH EMG activity in CIA-treated rats compared to controls is not driven or determined by local airway reflexes (ie, compensatory mechanisms). We propose that elevated baseline dilator EMG activity represents the persistent facilitation of cranial motor output as a result of CIA-induced plasticity in the CNS, preferentially increasing hypoglossal motor output. This could occur, for example, as a result of enhanced serotonergic (and/or other) facilitation of respiratory motor output, driving upper airway dilator muscle activity. LTF of respiratory motor output is serotonin-dependent.\(^{13–16,27}\) Furthermore, the withdrawal of the serotonergic excitatory drive has been implicated in sleep-related decrements of upper airway muscle activity, suggesting an important role for serotonin in the maintenance of upper airway patency across the sleep-wake cycle.\(^{28–31}\)

Indeed, it has been suggested that serotonin is involved in the augmented activity of upper airway dilator muscles during wakefulness in the English bulldog.\(^{31}\) At this time, it is not clear whether the elevated baseline SH EMG activity in CIA-treated rats has functional physiologic consequences or whether it is in any way advantageous to the animal (eg, a compensatory mechanism). We propose that the changes are reflective of altered CNS neurotransmodulation of cranial motor output that appear to be disadvantageous, at least in terms of reflex recruitment of upper airway dilator muscle activity in response to physiologic stimulation.

A further limitation of this study is the normalization of EMG data and our interpretation of these results. Since the actual electrical signal is not an appropriate index for comparison between groups, expressing baseline EMG activity relative to peak activity during hypercapnia allowed us to compare resting baseline EMG activities between the two groups. Peak activity during hypercapnia, however, may not always be truly representative of maximum EMG activity. For example, genioglossus EMG activity in humans is greatest during voluntary maneuvers, and this level of activity is considerably greater than that evoked by chemoreceptor stimulation.\(^{11}\) We chose to express baseline EMG activity relative to peak activity during hypercapnia because of concerns in expressing data relative to nonrespiratory-related activities such as swallowing. We acknowledge that this does not necessarily represent a normalization to maximum EMG activity, but rather a normalization to a reference stimulus. A major concern with the latter procedure, however, is that a decrease in the SH EMG response to hypercapnic stimulation would artifactually result in an increase in the relative measure of baseline EMG activity, and thus comparisons across groups may not be appropriate. Because of these concerns, although they have not been tested systematically, baseline SH EMG data also were expressed relative to the maximum activity encountered during a given experiment. Using this approach, we confirmed the finding of elevated baseline SH EMG in CIA-treated rats compared to controls.

Another limitation of this study is that the experiments were performed under anesthesia, which is known to have a preferentially depressant effect on upper airway dilator muscle activity compared to the DIA. However, comparisons were made between two groups of rats that underwent similar treatment and experimental protocols (including anesthesia). As such, the effects of anesthesia on baseline EMG activity and EMG responses to chemoreceptor stimulation should be comparable across groups, assuming no interactive effect of pentobarbitone anesthesia in CIA-treated rats alone. There are always additional concerns with interpreting data derived from experimental animals (especially under anesthesia) and applying these findings in terms of their physiologic and pathophysiologic significance to the conscious human.

In conclusion, our results show that CIA augments baseline upper airway dilator muscle activity and impairs upper airway EMG responses to acute hypoxia and asphyxia. We propose that CIA contributes to the pathophysiology of sleep-disordered breathing.

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