Po_2-dependent Changes in Intrinsic and Extrinsic Tongue Muscle Activities in the Rat

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Rationale: **Historically, respiratory-related research in sleep apnea has focused exclusively on the extrinsic tongue muscles (i.e., genioglossus, hyoglossus, and styloglossus). Until recently, the respiratory control and function of intrinsic tongue muscles (i.e., inferior and superior longitudinalis, transverses, and verticalis), which comprise the bulk of the tongue, were unknown.** ***Objectives:*** **The current study sought to determine if extrinsic and intrinsic tongue muscles are coactivated in conditions of hypoxemia comparable to that experienced by adults with obstructive sleep apnea.** ***Measurements:*** **Esophageal pressure and EMG activity of an extrinsic (hyoglossus) and an intrinsic (superior longitudinal) tongue muscle were studied in anesthetized, tracheotomized, spontaneously breathing rats. Average EMG activity was compared in a control gas condition (Pa_{O_2}, 160 ± 12 mm Hg) and in mild isocapnic hypoxia (Pa_{O_2}, 69 ± 7.2 mm Hg), with and without brief (3-breath) airway occlusions, pre- and postbilateral vagotomy.** ***Main Results:*** **(*1*) intrinsic and extrinsic tongue muscles are coactivated in mild hypoxia, (*2*) airway occlusion increased the activities of intrinsic retractor muscles in mild hypoxia, and (*3*) extrinsic retractor muscles have a steeper rate of rise of activity and an earlier burst onset relative to intrinsic retractor activities in mild hypoxia.** ***Conclusions:*** **These findings support our working hypothesis that airway patency is maintained not simply by activation of extrinsic tongue muscles but by the coactivation of intrinsic and extrinsic protrudor and retractor muscles.**

Keywords: EMG; hypoxia; sleep apnea

The tongue participates in a range of complex oromotor behaviors, including mastication, swallowing, and respiration. Functional deficits in tongue movement contribute to a host of disorders, including obstructive sleep apnea (1), dysarthria, and dysphagia (2–4). To date, studies that have examined the respiratory-related control of the tongue have focused primarily on the extrinsic tongue protrudor muscle, the genioglossus. As a result of this work, we know that the genioglossus is phasically active during the respiratory cycle and that its discharge onset precedes the onset of inspiratory airflow, stabilizing the airway for the negative pressure generated by the diaphragm (5–7). Although the genioglossus muscle is an important tongue muscle, it is only one of eight paired muscles that comprise the bulk of the tongue (8). Recent work in mammals has shown that the genioglossus and tongue retractor muscles are coactive in eupnea and hypercapnia (9–12), and that this coactivation improves pharyngeal airway patency (13, 14). These findings confirm that numerous tongue muscles are involved in the maintenance of airway patency, consistent with the muscular hydrostat theory of tongue motor control (15).

Evidence of intrinsic tongue muscle activities in hypercapnia indicates that these muscles also have the potential to contribute to lingual stiffness and airway reopening by virtue of their extensive interdigitation with extrinsic tongue muscles (9, 16). However, whether intrinsic and extrinsic tongue muscle coactivation occurs in hypoxemia is unknown. Accordingly, the current study compared the respiratory-related EMG activities of two tongue retractor muscles, one intrinsic (superior longitudinalis) and one extrinsic (hyoglossus) in mild hypoxia. Unlike previous studies of this type (17, 18), we manipulated the inspired gas levels to more closely approximate the hypoxemia experienced by individuals with obstructive sleep apnea (19, 20). Remarkably, we demonstrate coactivation of intrinsic and extrinsic tongue retractor muscles under these conditions, providing additional support for the muscular hydrostat model of tongue muscle function proposed by Kier and Smith (15) more than 20 years ago.

METHODS

Animals and Surgical Procedures

Procedures adhered to the guidelines established by the Institutional Animal Care and Use Committee at the University of Arizona. Ten male Sprague-Dawley rats (250–300 g) were used in the experiments. Animals were anesthetized via an intraperitoneal injection of urethane (1.3 g kg^{-1}). Additional details regarding the surgical preparation are provided in an online supplement.

Polyethylene catheters were placed in a femoral vein and a carotid artery for administration of intravenous fluids and blood gas sampling, respectively. The carotid artery catheter was connected to a pressure transducer (Grass-Telefactor PT300; Grass Instruments Co., Quincy, MA) for blood pressure monitoring. A saline-filled catheter was inserted into the esophagus and positioned at heart level for measurement of esophageal pressure (Pes). The trachea was cannulated and the animal breathed spontaneously. A constant flow of gas was directed across the inlet of the tracheotomy tube via a "t tube," as described previously (10) and outlined in an online supplement.

EMG Recordings

EMG recordings were obtained using fully insulated stainless steel fine-wire electrodes (0.002-mm diameter; California Fine Wire, Grover Beach, CA). Methodologies for obtaining intrinsic and extrinsic tongue muscle EMG activities and off-line treatment of EMG recordings are provided in an online supplement.

Experimental Protocol

Rats breathed 30% O_2, balance N_2 under control gas conditions and 16% O_2, 3% CO_2, balance N_2 in mild hypoxia. The effects of lung inflation were assessed in each condition by occluding the tracheotomy tube at end expiration for three consecutive respiratory cycles. At least three such occlusion maneuvers were attempted, with approximately 10 breaths between each maneuver. Animals were subsequently vagotomized, and the protocol was repeated. A three-step denervation procedure was conducted at the conclusion of each experiment to document EMG electrode placement. Details regarding the denervation protocol are provided in the online supplement.

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Data Analysis

Data were acquired using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Off-line analyses of EMG activity, Pes, mean arterial pressure, breathing frequency (f_R), and minute Pes ($f_R \times$ Pes) were performed using customized computer programs (Spike2). Average integrated EMG (iEMG) activity was defined as the area under the rectified signal divided by the burst duration (13, 21). In view of the profound increase in upper airway activities after vagotomy, the average iEMG activity in the vagi-intact animal was computed as a percentage of the maximal response recorded during the prevagotomy protocol. After vagotomy, a new maximum was computed as a percentage of the maximal response recorded at any time during the protocol. Details regarding the method used to calculate the average rate of rise of the EMG, and the EMG burst onset relative to the onset of inspiratory pressure development, are provided in an online supplement.

Inferential Statistical Analysis

Differences in average iEMG activity, onset time, and rate of rise for extrinsic and intrinsic tongue muscles between treatment conditions (control gas vs. mild hypoxia) before and after bilateral vagotomy were determined by repeated-measures analysis of variance. Significant effects were tested where appropriate using pairwise contrasts with Bonferroni adjustment.

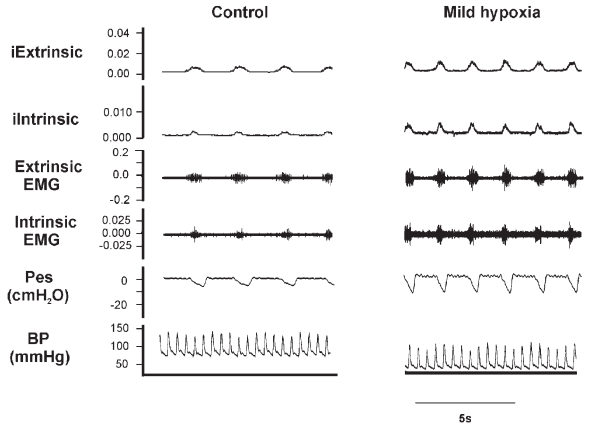

Figure 1. Representative integrated (i) and raw EMG recordings of extrinsic and intrinsic tongue muscles, esophageal pressures (Pes), and arterial blood pressure (BP) in a vagi-intact animal obtained under control gas conditions (30% O_2, 0% CO_2, balance N_2) and mild hypoxia (16% O_2, 3% CO_2, balance N_2).

RESULTS

Mean data describing mean arterial pressure, arterial pH, Pa_{CO_2}, Pa_{O_2}, f_R, and average Pes for control gas and mild hypoxia in vagus-intact preparations are given in the left two columns of Table 1.

Figure 1 shows representative recordings of extrinsic and intrinsic tongue muscle EMG activities, Pes, and blood pressure obtained from one animal under eupneic and mild hypoxic conditions. In this animal, inspiratory-related extrinsic and intrinsic activities were evident in eupnea; this was the case for 6 of 10 animals studied. Mild hypoxia was associated with progressive increases in EMG burst amplitude and breathing frequency and more negative inspiratory Pes swings. In this instance, 16% O_2 elicited increases in both phasic and tonic intrinsic EMG activities.

Figure 2 shows representative integrated intrinsic and extrinsic tongue muscle EMG bursts, with and without airway occlusion, under the control gas condition and in mild hypoxia. Closer inspection of the waveforms shows that burst envelopes for each condition do not diverge until well into inspiration. However, the amplitude of the occluded burst is larger, showing that lung inflation normally inhibits peak burst amplitude with little effect on the initial rate of rise activity.

Group data for minute Pes and average iEMG as a function of Pa_{O_2} and airway occlusion are shown in Figure 3. Minute Pes increased in hypoxia in cycles with and without airway occlusion. However, airway occlusion alone profoundly affected Pes, consistently doubling the average minute value under both control gas and hypoxic conditions ($p < 0.01$). Extrinsic and intrinsic activities also increased with hypoxia and exhibited a trend toward a further increase in amplitude with airway occlusion. The effects of airway occlusion were modest and of comparable magnitude for both extrinsic and intrinsic tongue muscle activities.

Average burst onset times for extrinsic and intrinsic tongue muscle activities are shown in Figure 4. Tongue muscle activities were consistently in advance of the inspiratory Pes swing in eupnea and hypoxia ($p < 0.01$), but there were no significant differences in the burst onset of extrinsic and intrinsic muscles, nor were there any significant changes with hypoxia.

Group results for mean rate of rise of extrinsic and intrinsic tongue muscle activities and Pes are shown in Figure 5. The rate of rise of tongue muscle activities did not change with mild hypoxia despite a significant increase in the rate of rise of minute Pes. The rate of rise of extrinsic activities was significantly greater than for intrinsic activities under all conditions.

Vagotomy halved respiratory frequency and increased average inspiratory Pes by 30% (Table 1). Nevertheless, minute Pes declined, resulting in hypoventilation (5–6 mm Hg increase in

TABLE 1. MEAN (AND SD) VALUES (OBTAINED AT MINUTE 4) FOR ARTERIAL PRESSURE, ARTERIAL pH, Pa_{CO_2}, Pa_{O_2}, BREATHING FREQUENCY, AND AVERAGE ESOPHAGEAL PRESSURE IN THE CONTROL GAS CONDITION (30% O_2, BALANCE N_2) AND IN MILD HYPOXIA (16% O_2, 3% CO_2, BALANCE N_2) BEFORE AND AFTER BILATERAL VAGOTOMY

	Prevagotomy		Postvagotomy	
Condition	Control	Mild Hypoxia	Control	Mild Hypoxia
MAP	96.04 (14.1)	63.17 (9.70)	86.83 (14.8)	68.9 (12.8)
pHa	7.35 (0.02)	7.36 (0.03)	7.33 (0.03)	7.34 (0.05)
Pa_{CO_2}	40.8 (2.5)	41.1 (2.6)	46.4 (2.9)	45.1 (4.1)
Pa_{O_2}	161 (12.7)	69.0 (7.2)	165 (11.5)	60.1 (8.0)
f_R, bpm	89.3 (18.8)	109.5* (23.8)	43.9 (8.9)	57.2* (7.7)
Pes, cm H_2O	−6.07 (0.85)	−8.23* (1.44)	−8.51 (1.55)	−12.44* (2.98)

Definition of abbreviations: bpm = breaths per minute; f_R = breathing frequency; MAP = mean arterial pressure; Pes = esophageal pressure; pHa = arterial pH.

* Indicates a significant difference ($p < 0.05$) between gas conditions within a treatment (i.e., pre- and postvagotomy).

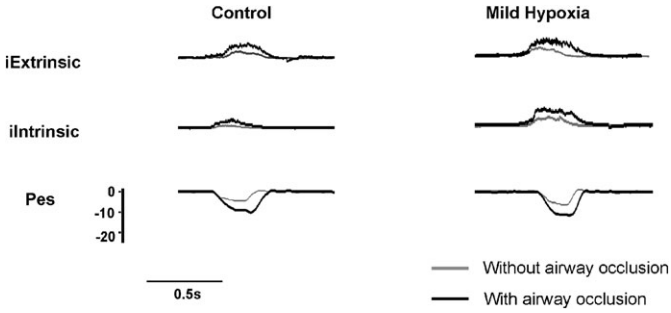

Figure 2. Representative integrated EMG recordings of extrinsic and intrinsic tongue muscles and Pes in a vagi-intact animal in the control gas condition and mild hypoxia, comparing burst envelopes with (*black traces*) and without (*gray traces*) airway occlusion.

Pa_{CO_2}). Vagotomy was associated with an almost threefold increase in average iEMG activity, significantly earlier onset times (0.15–0.2 seconds), and significantly faster ($\sim$40%) rates of rise of both the upper airway muscles and Pes relative to the prevagotomy control gas condition ($p < 0.01$). As shown in Figure 6, extrinsic activities were significantly in advance of intrinsic activities in the postvagotomy control gas condition ($p < 0.01$); however, this difference diminished in mild hypoxia with extrinsic activities leading intrinsic activities by 0.10 to 0.15 seconds. The rate of rise of intrinsic and extrinsic activities was significantly greater in mild hypoxia relative to the control gas conditions after vagotomy ($p < 0.01$ for both; Figure 7).

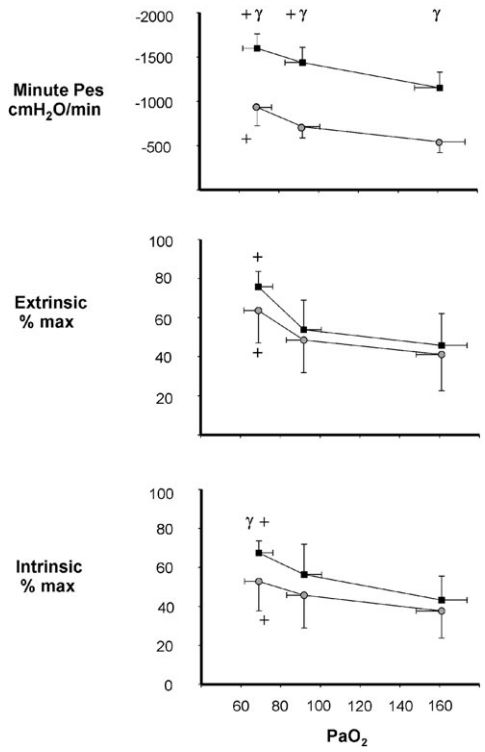

Figure 3. Mean (and SD) minute Pes (cm H_2O/minute) and extrinsic and intrinsic EMG activities (% of max) with (*black squares*) and without (*gray circles*) airway occlusion as a function of Pa_{O_2}. $^{+}$Indicates a significant difference ($p < 0.01$) for a given variable relative to the control gas condition. $^{\gamma}$ Indicates a significant difference ($p < 0.016$) between occlusion and no-occlusion conditions for a given variable.

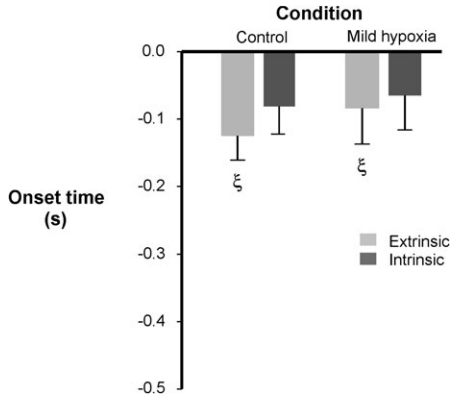

Figure 4. Mean (and SD) onset time (seconds) of extrinsic (*light gray*) and intrinsic (*dark gray*) tongue muscle activities under control gas conditions and in mild hypoxia in vagi-intact preparations. $^{\xi}$Indicates a significant difference ($p < 0.01$) in the onset of extrinsic tongue muscle activities relative to the onset of inspiration (denoted by the negative phase of the Pes swing).

DISCUSSION

Summary

Previous findings in the spontaneously breathing rat confirmed that intrinsic tongue muscle activities are often quiescent in eupnea but are coactivated with extrinsic tongue muscles in hypercapnia (9). The current findings build on this previous work and yield three important new observations. First, even very modest hypoxia leads to increases in the activities of both intrinsic and extrinsic tongue retractor muscles. Second, in hypoxia, airway occlusion augments the activity of both intrinsic and extrinsic tongue muscles. And third, extrinsic tongue muscle activity commences before intrinsic tongue muscle activity and is characterized by a steeper rate of rise.

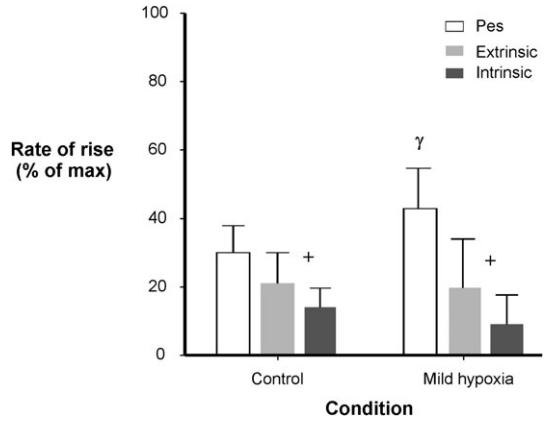

Figure 5. Mean (and SD) rate of rise (% of max) of Pes (*white*), extrinsic (*light gray*), and intrinsic (*dark gray*) tongue muscle activities in the control gas condition and mild hypoxia in vagi-intact preparations. $^{+}$Indicates a significant difference ($p < 0.01$) in rate of rise of extrinsic versus intrinsic tongue muscle activities under a given condition. $^{\gamma}$Indicates a significant difference in the rate of rise of Pes with respect to the control gas condition.

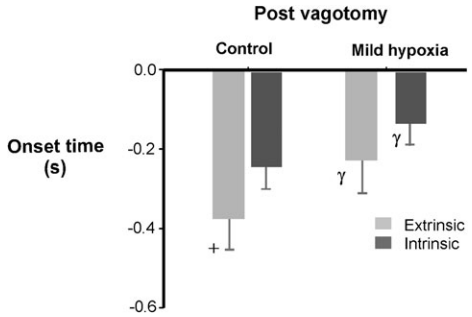

Figure 6. Mean (and SD) onset time of extrinsic (*light gray*) and intrinsic (*dark gray*) tongue muscle activities in the control condition and mild hypoxia postvagotomy. $^{+}$Indicates a significant difference ($p < 0.01$) in onset time of extrinsic versus intrinsic tongue muscle activities in a given condition. $^{\gamma}$Indicates a significant difference for a given variable with respect to the control gas condition.

Methodology

We recorded from an extrinsic retractor muscle (hyoglossus) and an intrinsic retractor muscle (superior longitudinalis), both of which are innervated by the lateral division of the hypoglossal nerve. Recent anatomic work in the rat has shown that the superior longitudinalis muscle runs in the midline along the long axis of the tongue (22). This anatomic arrangement has made it possible to isolate the muscle fiber bundles of the superior longitudinalis, and to sample their activity (9). Second, the nerve fiber branching pattern of the lateral division of the hypoglossal nerve permits the selective denervation of intrinsic tongue muscles as required under the current protocol, as shown previously (9), and in the online supplement (*see* Figure E1 in the online supplement).

We manipulated the inspired gases to achieve a level of hypoxemia reported during sleep in individuals with obstructive sleep apnea (19), and during voluntary breath holding in healthy individuals (23). In this respect, the present protocol contrasts markedly with previous studies that examined tongue muscle activities in severe hypoxemia (i.e., $Pa_{O_2} \sim 30$–45 mm Hg) and over extended time frames (i.e., $\sim$ 10-minute duration) (18, 24–26). Importantly, even very mild hypoxia produced a net increase in minute Pes and resulted in parallel increases in phasic intrinsic and extrinsic tongue retractor EMG activities. Under these same conditions, we observed marked differences in the timing of tongue muscle activities relative to Pes such that intrinsic and extrinsic retractor muscles were consistently coactivated in advance of the inspiratory pump muscles.

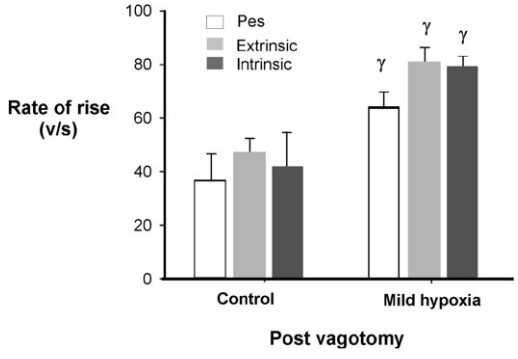

Figure 7. Mean (and SD) of rate of rise (% of max) of Pes (*white*) and extrinsic (*light gray*) and intrinsic (*dark gray*) tongue muscle activities in the control gas condition and mild hypoxia postvagotomy. $^{\gamma}$ Indicates a significant difference for a given variable with respect to the control gas condition.

Influence of Airway Occlusion

Consistent with previous results, the transient removal of phasic volume feedback via inspiratory airway occlusion increased the drive to pump (Pes) and extrinsic tongue muscles and slowed breathing frequency (9, 10, 21, 27). The present findings extend these observations to one of the intrinsic tongue muscles. In the vagus-intact preparation, airway occlusions increased the magnitude of intrinsic and extrinsic activities in both the control gas condition and mild hypoxia. Furthermore, the shape of both iEMGs was generally similar, showing a rapidly augmenting phase followed by a plateau and a decrementing phase. However, the magnitude of the increase in intrinsic tongue muscle EMG activities as a result of airway occlusion at end-expiration was significantly greater in mild hypoxia compared with the control gas condition (i.e., as shown in Figure 3). The extent to which such vagal influences may be expected to affect tongue muscle activities in human subjects is less clear, however, for although humans have functioning pulmonary stretch receptors, during quiet breathing their activity is believed to have little or no influence on inspiratory motor output (28, 29). Nevertheless, this is an important observation because it may indicate a preferential, lung inflation-mediated suppression of intrinsic versus extrinsic tongue muscle activities when the drive to breathe increases, such as in mild hypoxia or hypercapnia.

Vagotomy

Vagotomy was associated with profound increases ($\sim$ threefold) in upper airway EMG activities (9, 30, 31). The failure of intrinsic and extrinsic EMG activities to increase in response to airway occlusion after vagotomy indicates that pulmonary afferents with axons in the vagus nerves mediate these reflex changes in upper airway activities (32). Of importance for the present study, extrinsic tongue muscle activation consistently commenced before intrinsic activities under all conditions. The absence of any such onset differences before vagotomy (with or without airway occlusion) suggests a differential modulation of intrinsic versus extrinsic tongue muscles by tonic vagal inputs.

Physiologic Significance

Although the tongue comprises both intrinsic and extrinsic tongue muscles, respiratory-related research has focused largely on the extrinsic tongue musculature and its role in the genesis of obstructive sleep apnea. This focus is surprising given previous studies that indicate intrinsic tongue muscles have the potential to affect the mechanical actions of the extrinsic tongue muscles (15, 22, 33, 34) and thus to make a significant contribution to lingual stiffness (35). The results of the present study in anesthetized rats reveal that intrinsic tongue muscles are coactivated with extrinsic tongue muscles in eupnea and in mild hypoxia, and that both intrinsic and extrinsic tongue muscle activities increase when the airway is occluded. These are important findings that bear directly on individuals with sleep apnea who experience episodic airway obstruction coupled with hypoxemia and hypercapnia. On the basis of our current observations and previous findings in hypercapnia (9), we hypothesize that airway obstruction may be associated with brisk increases in the activity of both extrinsic and intrinsic tongue muscles as a result of the removal of lung volume–mediated inhibition, with subsequent increases in activities the result of excitatory inputs from periph-

eral and central chemoreceptors responding to hypoxic and hypercapnic blood.

Conflict of Interest Statement: E.F.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; P.L.J. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; R.F.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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