Coordination of intrinsic and extrinsic tongue muscles during spontaneous breathing in the rat

E. F. Bailey and R. F. Fregosi

Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona 85721-0093

Submitted 15 July 2003; accepted in final form 8 September 2003

Bailey, E. F., and R. F. Fregosi. Coordination of intrinsic and extrinsic tongue muscles during spontaneous breathing in the rat. J Appl Physiol 96: 440–449, 2004. First published October 3, 2003; 10.1152/japplphysiol.00733.2003.—The muscular-hydrostat model of tongue function proposes a constant interaction of extrinsic (external bony attachment, insertion into base of tongue) and intrinsic (origin and insertion within the tongue) tongue muscles in all tongue movements (Kier WM and Smith KK. Zool J Linn Soc 83: 207–324, 1985). Yet, research that examines the respiratory-related effects of tongue function in mammals continues to focus almost exclusively on the respiratory control and function of the extrinsic tongue protrusor muscle, the genioglossus muscle. The respiratory control and function of the intrinsic tongue muscles are unknown. Our purpose was to determine whether intrinsic tongue muscles have a respiration-related activity pattern and whether intrinsic tongue muscles are coactivated with extrinsic tongue muscles in response to respiratory-related sensory stimuli. Esophageal pressure and electromyographic (EMG) activity of an extrinsic tongue muscle (hyoglossus), an intrinsic tongue muscle (superior longitudinal), and an external intercostal muscle were studied in anesthetized, tracheotomized, spontaneously breathing rats. Mean inspiratory EMG activity was compared at five levels of inspired CO2. Intrinsic tongue muscles were often quiescent during eupnea but active during hypercapnia, whereas extrinsic tongue muscles were active in both eupnea and hypercapnia. During hypercapnia, the activities of the airway muscles were largely coincident, although the onset of extrinsic muscle activity generally preceded the onset of intrinsic muscle activation. Our findings provide evidence, in an in vivo rodent preparation, of respiratory modulation of motoneurons supplying intrinsic tongue muscles. Distinctions noted between intrinsic and extrinsic activities could be due to differences in motoneuron properties or the central, respiration-related control of each motoneuron population.

hydrostat; hyoglossal; electromyogram

THE TONGUE PARTICIPATES IN A wide variety of oromotor behaviors, which include mastication, swallowing, and respiration. The tongue lacks an internal bony skeleton and is composed almost entirely of muscle. Muscle tissue comprises primarily aqueous liquid and is virtually incompressible. Thus contraction of a muscle does not significantly change tongue volume. Instead, the tension produced by contraction is transmitted as an increase in pressure. This physical arrangement permits the musculature of the tongue itself to create motion and provide mechanical support for that motion (34).

Tongue musculature comprises intrinsic (originate and terminate within the tongue) and extrinsic (an external bony origin and insertion into the tongue base) muscle fibers. There are four orthogonally related intrinsic tongue muscles (vertica]
METHODS

Animals and surgical procedure. All procedures adhered to the guidelines established by the Institutional Animal Care and Use Committee at the University of Arizona. Ten male Sprague-Dawley rats weighing 250–350 g were used in the experiments. Animals were induced with isoflurane (2–2.5%) and subsequently anesthetized via an intraperitoneal injection of urethane (1.3 g/kg). This anesthetic was selected because it has been shown to preserve the response to CO₂ (16) and Breuer-Hering reflexes (19). No surgical procedures were performed until animals were unresponsive to a strong paw pinch with a hemostat. Paw pinch was also used to assess the need for additional anesthesia (0.3 g/kg) at regular intervals. Animals were supine with limbs secured to the operating table throughout the experiment. Rectal temperature was maintained at 37°C with a thermistor connected to a servo-controlled heating pad (model D1-L, Haake). At the end of the experiment, animals were euthanized by an intravenous dose of pentobarbital sodium.

Polyethylene catheters were placed in a femoral vein and artery and the esophagus. The femoral vein catheter (PE-50) permitted administration of intravenous fluids throughout the experiment. The arterial catheter (PE-50) was connected to a pressure transducer (model PT300, Grass-Telefactor) for blood pressure monitoring. The esophageal catheter (PE-160) was filled with saline, positioned at heart level, and connected to a pressure transducer (model Y94-21, Coulbourn Instruments) for measurement of esophageal pressure. The trachea was cannulated caudal to the larynx (PE-240), and the animal breathed spontaneously. The vagus nerves were isolated and looped with suture for sectioning later in the protocol.

A constant flow of gas was directed across the inlet of the tracheotomy tube via a T tube. Mixtures of O₂, CO₂, and N₂ were delivered to the animal by connecting the outlet port of the rotameter to the T tube as described previously (15). Inspired gas concentrations were monitored with CO₂ (Applied Electrochemistry, model OM-11) and O₂ analyzers (Beckman, model OM-11). Arterial blood samples (0.4-ml aliquots) were taken to determine the partial pres.

All procedures adhered to the guidelines established by the Institutional Animal Care and Use Committee at the University of Arizona. Ten male Sprague-Dawley rats weighing 250–350 g were used in the experiments. Animals were induced with isoflurane (2–2.5%) and subsequently anesthetized via an intraperitoneal injection of urethane (1.3 g/kg). This anesthetic was selected because it has been shown to preserve the response to CO₂ (16) and Breuer-Hering reflexes (19). No surgical procedures were performed until animals were unresponsive to a strong paw pinch with a hemostat. Paw pinch was also used to assess the need for additional anesthesia (0.3 g/kg) at regular intervals. Animals were supine with limbs secured to the operating table throughout the experiment. Rectal temperature was maintained at 37°C with a thermistor connected to a servo-controlled heating pad (model D1-L, Haake). At the end of the experiment, animals were euthanized by an intravenous dose of pentobarbital sodium.

Polyethylene catheters were placed in a femoral vein and artery and the esophagus. The femoral vein catheter (PE-50) permitted administration of intravenous fluids throughout the experiment. The arterial catheter (PE-50) was connected to a pressure transducer (model PT300, Grass-Telefactor) for blood pressure monitoring. The esophageal catheter (PE-160) was filled with saline, positioned at heart level, and connected to a pressure transducer (model Y94-21, Coulbourn Instruments) for measurement of esophageal pressure. The trachea was cannulated caudal to the larynx (PE-240), and the animal breathed spontaneously. The vagus nerves were isolated and looped with suture for sectioning later in the protocol.

A constant flow of gas was directed across the inlet of the tracheotomy tube via a T tube. Mixtures of O₂, CO₂, and N₂ were delivered to the animal by connecting the outlet port of the rotameter to the T tube as described previously (15). Inspired gas concentrations were monitored with CO₂ (Applied Electrochemistry, model OM-11) and O₂ analyzers (Beckman, model OM-11). Arterial blood samples (0.4-ml aliquots) were taken to determine the partial pressures of O₂ and CO₂ (PACO₂), pH, and base excess with an Instrumentation Laboratories blood-gas analyzer (model 1640). Base deficits were corrected by intravenous administration of sodium bicarbonate.

Electromyogram recordings. All electromyogram (EMG) recordings were obtained with the use of stainless steel fine-wire electrodes measuring 0.002 mm in diameter (California Fine Wire). Electrodes were fully insulated along their length; i.e., the electrode tip was the only active pick-up area. EMG recording of the intrinsic tongue retrusor muscle (superior longitudinal) was obtained by inserting two recording electrodes (2 mm apart) into the dorsum of the tongue in the midline, anterior to the premolar eminence, ~0.8 cm from the tongue tip. The extrinsic tongue retrusor muscles (hyoglossus) were exposed with a ventral approach (18). The external intercostal muscles were exposed by removal of muscle overlying the fourth and fifth intercostal spaces (4). Two electrodes subsequently were inserted into the bellies of each of these muscles. Correct electrode placement for the extrinsic tongue retrusor muscles was confirmed by connecting the inserted electrodes to a stimulator (model S48, Grass) via a stimulus isolation unit (model SIU7C, Grass). Current (0.1–0.15 mA) was passed through the wires (60 Hz, 500 ms), and the direction of tongue movement was observed to ensure that the tongue retracted with stimulation (15). Alternating-current differential amplifiers (model 7WU16K, Grass) were used to amplify and filter (30–3,000 Hz) the EMG. Tongue muscle EMG activities were sampled at 10 kHz and intercostal EMG activities at 5 kHz. The amplified and filtered EMG signals were rectified and moving-time averaged with a time constant of 200 ms.

Experimental protocol. Each animal was exposed successively to five levels of hypoxic hypercapnia [inspired CO₂ fraction (FiCO₂) = 0, 0.3, 0.6, 0.9, and 0.12; inspired O₂ = 0.35; balance N₂]. Each level was maintained for a minimum of 5 min. The effects of vagal inhibition secondary to lung inflation were assessed under each gas condition by occluding the tracheotomy tube at end expiration for two to three respiratory cycles (4). At least three occlusions were attempted at each level with ~10 breaths between successive occlusions. The animals subsequently were bilaterally vagotomized at the midcervical level and the inspired gas protocol repeated. Hyoglossal nerve denervations were completed at the termination of each experiment as outlined below.

Denervation protocol. To confirm that intrinsic tongue muscle EMG activities were not contaminated by volume conductance of electrical activity from adjacent muscles, a three-step denervation procedure was conducted at the conclusion of each experiment. It is important to note that the denervation studies were conducted under hypercapnia conditions so that any change of volume conductance would be amplified. In this way, elimination of activity after nerve branch section allowed us to confidently conclude that electrode placement was optimal. The denervation protocol was based on a previously published detailed description of the anatomy of the hyoglossal nerve in the rat (28). The results of the denervation protocol, in a representative animal, are shown in Fig. 1. Figure 1A shows the EMG activities with intact hyoglossal nerves. Figure 1B shows EMG activities after the bilateral section of the medial hyoglossal nerve branches at the point of bifurcation of lateral and medial hyoglossal nerve branches. Note that the activities do not change as a result of the transection, which eliminates tongue protrusor muscle activities (i.e., transversus, verticalis, and genioglossus muscles). The lateral hyoglossal nerve branches subsequently were sectioned bilaterally, ~8 mm distal to the point of bifurcation of the medial and lateral hyoglossal nerve branches. This section eliminated only intrinsic tongue retrusor activities (i.e., superior and inferior longitudinal muscles), leaving extrinsic re
trusor activities intact (Fig. 1C).

In the event that 1) either intrinsic or extrinsic re
trusor tongue muscle EMG activities diminished after section of the medial hyoglossal nerve branches and/or 2) there was a persistence of EMG activities after whole hyoglossal nerve section, it was presumed that EMG electrode placement was inaccurate and the record was excluded from subsequent analysis.

Data analysis. All data were acquired by using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Subsequent offline analyses of EMG activity and esophageal pressure were performed using customized computer programs (Spike2).

Mean EMG activity was defined as the area under the rectified signal divided by the burst duration. Mean EMG activity was ex
dressed as a percentage of the maximal response, which was defined as the highest level of activity observed during the protocol (typically at 9–12% FICO₂ postvagotomy). The relative efficacy of lung volume feedback in inhibiting EMG activity at any given level of FICO₂ was estimated as follows

\[
\text{% Increase in EMG as a result of end-expiratory airway occlusion} = \frac{(\text{EMG during occluded breath}) - \text{(EMG during unoccluded breath)}}{\text{EMG occluded breath}} \times 100
\]

This calculation was used to assess the increase in EMG activity evoked by end-expiratory airway occlusions at each level of FICO₂.

Temporal differences in the onset of intrinsic, extrinsic, and intercostals activities were determined relative to the downward (negative) deflection of the esophageal pressure record associated with each inspiratory effort. Differences were determined by visual inspection of the rectified and averaged signals corresponding to the unoccluded breaths. Average time to peak activity was determined by visual
Fig. 1. Representative raw intercostal, extrinsic (hyoglossus), and intrinsic (superior longitudinal) electromyogram (EMG) recordings and esophageal pressure (Pesp) and blood pressure (BP) during progressive denervation of the hypoglossal nerves (XII). A: postvagotomy activities in the hypoglossal intact preparation. B: activities after bilateral section of the medial (M) XII branches eliminating all tongue protrusor activities (i.e., genioglossus, transversus, and verticalis muscles). C: effect of sectioning the distal portions of the lateral (L) XII branches removing intrinsic retrusor activities (i.e., superior and inferior longitudinal muscles). D: elimination of extrinsic and intrinsic activities on bilateral sectioning of the whole hypoglossal nerves. Records displayed in A–D were sampled at 1, 3, 5, and 7 min postexposure to inspired CO2 fraction (FICO2) of 12%. Note that the amplitude of the intercostal and extrinsic tongue muscle activities declines in C and D. The decline is attributed to recovery associated with declining respiratory drive.

INFERENTIAL STATISTICAL ANALYSIS

Inferential statistical analysis. Differences in mean EMG activity, onset time, and time to peak were obtained from a series of 20 consecutive breaths collected in the fifth minute at each level of the CO2 stimulus pre- and postvagotomy.

Inferential statistical analysis. Differences in mean EMG activity, onset time, and time to peak for intrinsic, extrinsic, and external intercostal muscles within conditions (i.e., between successive levels of FICO2) and between conditions (i.e., pre- vs. postvagotomy) were determined by repeated-measures analysis of variance. Significant effects were tested where appropriate using pairwise contrasts with significance levels adjusted by the Bonferroni procedure (P < 0.002). Additional follow-up analyses included examination of the relative linearity of the response to CO2 pre- and postvagotomy for each muscle.

RESULTS

Effect of phasic lung inflation. Figure 2 shows representative recordings of intrinsic and extrinsic tongue muscle activities under baseline conditions with and without end-expiratory airway occlusion. End-expiratory occlusions resulted in increased burst amplitude and duration of extrinsic tongue muscle activities (i.e., extrinsic EMG activities were markedly suppressed by lung inflation). In this animal, end-expiratory occlusion increased the burst duration of intrinsic tongue muscle activities, although the effects on burst amplitude were not as pronounced. Despite some variability in the effects of end-expiratory occlusions on intrinsic activities at baseline and at 3% FICO2, no significant differences were detected in the effects of lung inflation on intrinsic vs. extrinsic tongue muscle activities.

Closer inspection of the rectified waveforms (Fig. 2B) shows that occluded and unoccluded bursts do not diverge until well into inspiration and that the burst envelope for each muscle remains similar up to the point of divergence. However, at the onset of lung inflation-mediated inhibition the waveforms clearly diverge, and the occluded cycles are prolonged and associated with more EMG activity. The changes in activity between occluded and unoccluded breaths were eliminated in the postvagotomy condition, confirming that this effect was mediated by vagal afferents.

Effect of hypercapnia and vagotomy. Figure 3 shows representative recordings of the external intercostal, extrinsic, and intrinsic tongue muscle EMGs, esophageal pressure, and blood pressure in a vagally intact animal at baseline and at 3% and 9% FICO2. Inspiratory-related phasic extrinsic and intercostal muscle activities were evident at all levels of the CO2 stimulus, whereas intrinsic tongue muscle activities emerged when the FICO2 level reached 6%. Subsequent bilateral vagotomy (Fig. 4) resulted in profound increases in phasic extrinsic and intrinsic tongue muscle activities relative to the prevagotomy condition. Increasing the level of FICO2 in this postvagotomy state augmented burst amplitude and duration in both the upper airway muscles with the greatest relative increase evident in intrinsic tongue muscle activities.

Group data for mean EMG activity as a function of inspired CO2 pre- and postbilateral vagotomy are shown in Fig. 5. Values are expressed as a percentage of the maximum mean EMG activity recorded during the experiment. In the prevagotomy condition, mean extrinsic (top, gray bars) and intercostal muscle activities (bottom) showed progressive increases with increasing levels of inspired CO2 as shown previously (4). In comparison, intrinsic tongue muscle activities (top, black bars) were significantly smaller in magnitude and were largely unaffected by increases in FICO2.

Mean intrinsic and extrinsic tongue muscle activities were significantly increased postvagotomy under baseline conditions and at all levels of the CO2 stimulus (P < 0.002). Conversely, the magnitude of intercostal activities did not change pre- vs. postvagotomy. Note that whereas upper airway activities plateaued at FICO2 levels in excess of 9%, the respiratory pump muscle activities increased with successive increases in respiratory drive. Thus upper airway muscles attained their maximal activation levels at lower levels of hypercapnia.
Figure 6 shows representative raw EMG recordings after unilateral and then bilateral vagotomy. Note the increase in amplitude of extrinsic activities with unilateral vagotomy, whereas the amplitude of intrinsic activities does not change. Thus intrinsic activities are "released" from inhibition only after sectioning both vagi. Whereas the amplitude of extrinsic activities stabilizes seconds after bilateral vagotomy, the burst amplitude of intrinsic activities evolves gradually. Seven of the ten animals studied exhibited comparable responses to vagal section. The amplitude of respiratory pump muscle activities (external intercostal) is minimally affected by either of the vagal sections, although breathing frequency slows considerably.

Effect on burst pattern and timing. Figure 7 shows representative records of the relative onset of intrinsic and extrinsic tongue muscle activities and intercostal activities pre- and post vagotomy. Extrinsic and intrinsic tongue muscle activities always preceded the negative phase of the esophageal pressure recording. This effect was augmented by increased CO₂. Importantly, bilateral vagotomy magnified the onset time difference between upper airway and respiratory pump muscles and between extrinsic and intrinsic tongue muscle activities. Note that in the postvagotomy condition extrinsic activities are phase spanning, i.e., bridging both the late expiratory and inspiratory phases of the respiratory cycle. The onset time differences between extrinsic and intrinsic activities remained stable or declined slightly with successive increases in FICO₂ (Fig. 8).

Group data for mean onset time of upper airway and respiratory pump muscles relative to the onset of the negative phase of the esophageal pressure record are shown in Fig. 8. As anticipated, intercostal activities coincided with the onset of the inspiratory esophageal pressure swing. By comparison, the onset of extrinsic activities was significantly in advance of both intercostal and intrinsic activities for inspired CO₂ levels exceeding 6% (P < 0.002). Vagotomy augmented this trend and increased the onset time difference between the intrinsic and extrinsic tongue muscles by as much as 0.4 s (range 0.1–0.4 s) (P < 0.002).

Time to peak activity of intrinsic and extrinsic tongue muscle activities is shown in Fig. 9. Intrinsic and extrinsic tongue muscles exhibited comparable times to peak activity under a given gas condition pre- and postvagotomy with the exception of the baseline postvagotomy condition in which intrinsic times to peak were significantly lower (P < 0.002). However, there were significant differences in the overall response to CO₂ for intrinsic vs. extrinsic times to peak in the postvagotomy condition. That is, the intrinsic tongue muscles time to peak activity increased with successive increases in FICO₂ whereas extrinsic times to peak activity declined over the same conditions (P < 0.001).

DISCUSSION

Summary. In this study, intrinsic tongue muscle activities were compared with the activities of an extrinsic tongue
Fig. 3. Representative raw EMG recordings of IIC and extrinsic and intrinsic tongue muscle activities, Pes, and arterial BP in 1 animal at 0, 3, and 9% \( \text{F}_{2\text{CO}} \) before bilateral vagotomy.

Fig. 4. Representative raw EMG recordings of IIC and extrinsic and intrinsic tongue muscle activities, Pes, and arterial BP in 1 animal at 3, 6, and 9% \( \text{F}_{2\text{CO}} \) postbilateral vagotomy. Note that EMG recordings displayed in this figure are displayed at \( \times 0.5 \) the magnification of those depicted in Fig. 3.
Fig. 5. EMG activities for intrinsic and extrinsic tongue muscles and external intercostal muscles in normocapnia and at each of 4 levels of $F_\text{ICO}_2$ (3, 6, 9, and 12%) pre- (A) and postbilateral vagotomy (B). Values are means ± SD. max, Maximum. ‡Significant difference between extrinsic tongue muscle activities pre- vs. postvagotomy at a given level of $F_\text{ICO}_2$, $P < 0.002$. *Significant difference between intrinsic tongue muscle activities pre- vs. postvagotomy at a given level of $F_\text{ICO}_2$, $P < 0.002$. *Significant differences between intrinsic and extrinsic activities at a given level of $F_\text{ICO}_2$ pre- and postvagotomy, $P < 0.002$.

Fig. 6. Extended recording (~4-min duration) showing representative raw EMG recordings of IIC, extrinsic tongue, and intrinsic tongue muscle activities and Pes before and after unilateral vagotomy and bilateral vagotomy. Note an increase in extrinsic activities with unilateral vagal section that is immediately augmented with bilateral vagal section. In comparison, intrinsic activities appear unaffected by unilateral section and then gradually evolve, reaching maximum amplitude over a minute later.
retrusor muscle and an external intercostal muscle at successive levels of inspired CO₂, with and without vagally mediated lung volume feedback. The main results may be summarized as follows. First, intrinsic tongue muscles have a respiration-related activity pattern, although they are often quiescent during eupnea but active during hypercapnia. Second, intrinsic and extrinsic tongue muscles are coactivated in response to respiratory-related sensory stimuli. Third, the inhibition of intrinsic tongue muscle activities by tonic vagal influences exceeds that of the extrinsic tongue muscles.

Critique of the method. As stated in the introduction, there are no reports that document the respiratory-related control and function of the intrinsic tongue muscles. This lack of information is in part attributable to the complex anatomy of intrinsic tongue muscles (1, 28, 35) that makes recording their isolated activities in vivo challenging. However, recent anatomic work has shown that superior longitudinal fibers run in the midline in the long axis of the rodent tongue (35). This anatomic arrangement makes it feasible to isolate the muscle fibers of the superior longitudinal muscle and to sample their activity. Nevertheless, we acknowledge that the complexity of the tongue anatomy and the proximity of adjacent musculature increase the likelihood of signal contamination via volume conductance of action potentials from adjacent musculature. To address this concern, selective nerve sectioning was conducted at the termination of each experiment (Fig. 1) to confirm that intrinsic activities were not a product of current spread from the adjacent extrinsic tongue musculature or from vicinity of nerve conduction. Additional analyses of this nature were not conducted.

Fig. 7. Onset times for intrinsic, extrinsic, and external intercostal muscles at 6% (A and C) and 9% FICO₂ (B and D) pre- (top) and postvagotomy (bottom) in a representative animal. Solid vertical lines, onset for intrinsic (I) and extrinsic (E) muscle activities; vertical dashed lines, onset of IIC activities. Rectified integrated intercostals (IIC) and extrinsic (iExtrinsic, black traces) and intrinsic (iIntrinsic, gray traces) EMG signals are displayed with respect to the Pes signal at each level of FICO₂. For purposes of visualization, the integrated intrinsic EMG record in A is displayed at ×3 the magnification of those shown in B–D. For all other signals, the scale is held constant between panels.

Fig. 8. Onset times for intrinsic, extrinsic, and IIC muscle activities under normocapnia and at 4 levels of FICO₂ (3, 6, 9, and 12%) pre- and postvagotomy. Values are means ± SD. Results are displayed with respect to the Pes swing coincident with the inspiratory phase of the respiratory cycle. ‡Significant difference between extrinsic tongue muscle activities pre- vs. postvagotomy, P < 0.002. *Significant difference between intrinsic tongue muscle activities pre- vs. postvagotomy, P < 0.002. #Significant difference between intrinsic and extrinsic activities at a given level of the CO₂ stimulus pre- and postvagotomy, P < 0.002. Note that no significant differences in onset time were detected for the IIC activities.
synergists with potential for respiratory-related activities, e.g., transversus, mylohyoid, or pharyngeal constrictor muscles. If the results of the denervation studies were equivocal, the data were excluded from all subsequent analyses.

Effects of hypercapnia. Previously, our laboratory documented the effects of hypercapnia on extrinsic tongue protrusor (genioglossus) and retrusor (hyoglossus) activities (4). It was concluded that tongue muscle activities are dominated by the inhibitory effects of lung inflation at low and moderate levels of hypercapnia but that the effects of chemoreceptor stimulation dominate when the $F_{\text{ICO}_2}$ level exceeds 6%. The present findings confirm the earlier findings for the extrinsic retrusor (hyoglossus) and demonstrate a distinction in the effect of chemoreceptor stimulation on intrinsic vs. extrinsic tongue muscle activity. Specifically, in vagi-intact animals, intrinsic activities are facilitated by chemoreceptor stimulation, but this facilitation appears to be overridden by the effects of phasic and tonic vagal inhibition at all but the highest levels of inspired $CO_2$.

Chemoreceptor stimulation had marked effects on the relative timing of tongue and intercostals muscle activity. Although similar timing differences have been observed by comparing phrenic and whole hypoglossal nerve discharges in the cat (21) and extrinsic tongue protrusor and retrusor activity in the rat (4), these results failed to discriminate between intrinsic and extrinsic motoneuronal activity. The present findings demonstrate for the first time that both extrinsic and intrinsic tongue retrusor activity advance relative to intercostal activity as chemoreceptor stimulation intensifies. Moreover, extrinsic tongue muscle activity occurs in advance of intrinsic activity under all conditions. Although the mechanism that underlies the difference in burst onset between intrinsic and extrinsic tongue muscles has not been established, the present results show clearly that lung inflation has no influence on burst onset, ruling out a role for pulmonary stretch receptors.

Phasic and tonic vagal modulation. In this study, the relative efficacy of phasic or lung inflation-mediated inhibition was assessed by tracheal occlusion at end expiration. Our findings confirm previously published results showing phasic, inspiratory-related extrinsic activities in eupnea, with increased activities on prevention of lung inflation (4). Importantly, the present results indicate that intrinsic activities are similarly modulated by phasic vagal feedback, although the effects are typically smaller in magnitude and more variable.

Although tracheal occlusion at end expiration eliminates lung inflation-mediated inhibition, there is good evidence that it is not effective in eliminating tonic vagal feedback (32, 39). To assess the significance of tonic vagal feedback, we compared extrinsic and intrinsic tongue muscle activities before and after bilateral vagotomy. Vagotomy resulted in profound changes in intrinsic burst amplitude, onset time, and time to peak activity (refer to Fig. 3), and these effects were magnified with successive increases in $CO_2$. Significant differences in the onset time and time to peak activity between intrinsic and extrinsic tongue muscle activities were also noted under these conditions. Thus tonic low-frequency discharge appears to play a more significant physiological role in modulating intrinsic activities than phasic vagal modulation via pulmonary stretch receptors. Furthermore, the comparatively small increase in intrinsic activities with increasing $F_{\text{ICO}_2}$ observed before vagotomy (see Fig. 5) suggests that tonic vagal modulation is a powerful modulator of intrinsic hypoglossal motoneuronal activities in the intact animal under conditions of respiratory challenge.

Vagal memory. In the present experiment, unilateral and bilateral vagotomy resulted in the immediate disinhibition of extrinsic tongue muscle activities. In contrast, intrinsic activities were not changed with unilateral vagotomy but gradually increased postbilateral vagotomy. The reasons for these phenomena are uncertain; however, several mechanisms may be postulated. First, the absence of an effect of unilateral vagotomy on intrinsic activities suggests some degree of redundancy in vagal inputs onto intrinsic hypoglossal motoneurons. The selectivity of the effect may be ascribed to differential vagal modulation of intrinsic and extrinsic hypoglossal motoneurons or to different neural circuits controlling extrinsic and intrinsic hypoglossal motoneuronal activity. The latter possibility is of particular interest in light of anatomic evidence that documents distinct medullary projections onto intrinsic vs. extrinsic hypoglossal motoneuron pools (36). Second, the patterns of intrinsic tongue muscle activities after vagotomy may be due to accommodation of intrinsic muscle motoneurons to the removal of vagal input or to the persistence of vagal
inhibition onto intrinsic hypoglossal motoneurons. There is good evidence that such a persistence or “memory” arises in pulmonary stretch receptors that effect a short- or intermediate-term inhibition of activities that lasts beyond the actual period of stimulation (13).

Physiological significance. The present findings confirm the respiratory modulation of intrinsic tongue muscle activities and the coactivation of intrinsic with extrinsic tongue muscles in hyperoxic hypercapnia in the spontaneously breathing rat. These findings are of significance because intrinsic and extrinsic tongue muscles are likely co-activated during airway obstruction. That is, both intrinsic and extrinsic tongue muscle activities will be augmented when the airway is obstructed at end expiration because phasic and tonic vagal inhibition are diminished and there is an increased drive to breathe. Moreover, because of the arrangement of intrinsic muscle fibers with respect to extrinsic tongue muscles, their coactivation has the potential to increase lingual stiffness and to contribute to airway reopening via tongue protrusion. Thus it is conceivable that airway patency is maintained not simply by tongue protrusion or by the coactivation of extrinsic tongue protrusor and retractor muscles (17, 25) but by the coactivation of intrinsic and extrinsic protrusor and retractor muscles.

Finally, the distinctions noted between intrinsic and extrinsic activities with respect to burst magnitude and onset time are of significance for they indicate that the properties of intrinsic and extrinsic hypoglossal motoneurons themselves differ and/or that the inputs controlling intrinsic and extrinsc motoneuronal activities differ. The latter suggestion is particularly relevant in light of recent anatomic evidence demonstrating distinct and shared inputs from premotoneurons in the medullary reticulum formation projecting onto intrinsic and extrinsic motoneurons pools within the hypoglossal nucleus (36). On the basis of these findings and the present observations it seems reasonable to suggest that results obtained from “hypoglossal” motoneurons or from the stimulation of the whole hypoglossal nerve should not be interpreted to represent the activities of extrinsic tongue muscles exclusively.

Conclusion. Understanding lingual behavior in breathing is of considerable importance because it plays a critical role in regulating upper airway resistance, and its dysfunction contributes in a major way to obstructive sleep apnea. Yet, to date, the respiratory role of the muscles that comprise the bulk of the tongue has largely been ignored. The present findings underscore the complexity of tongue movements and emphasize the need to further evaluate the role of the intrinsic lingual musculature in mechanisms of airway defense.

ACKNOWLEDGMENTS

The authors thank Dr. Andrew J. Fuglevand for critical review of the manuscript and Dr. Patricia Jones for assistance with the statistical analyses.

GRANTS

This study was supported by National Institutes of Health Grants DC-05728 and HL-56676.

REFERENCES


J Appl Physiol • VOL 96 • FEBRUARY 2004 • www.jap.org


