Anatomic consequences of intrinsic tongue muscle activation

E. Fiona Bailey,1 Yu-Hsien Huang,1 and Ralph F. Fregosi1,2

Departments of 1Physiology and 2Neurobiology, The University of Arizona, Tucson, Arizona

Submitted 29 March 2006; accepted in final form 28 June 2006

Anatomic consequences of intrinsic tongue muscle activation. J Appl Physiol 101: 1377–1385, 2006. First published July 6, 2006; doi:10.1152/japplphysiol.00379.2006.—We recently showed respiratory-related coactivation of both extrinsic and intrinsic tongue muscles in the rat. Here, we test the hypothesis that intrinsic tongue muscles contribute importantly to changes in velopharyngeal airway volume. Spontaneously breathing anesthetized rats were placed in a MRI scanner. A catheter was placed in the hypopharynx and connected to a pressure source. Axial and sagittal images of the velopharyngeal airway were obtained, and the volume of each image was computed at airway pressures ranging from +5.0 to −5.0 cmH2O. We obtained images in the hypoglossal intact animal (i.e., coactivation of intrinsic and extrinsic tongue muscles) and after selective denervation of the intrinsic tongue muscles, with and without electrical stimulation. Denervation of the intrinsic tongue muscles reduced velopharyngeal airway volume at atmospheric and positive airway pressures. Electrical stimulation of the intact hypoglossal nerve increased velopharyngeal airway volume; however, when stimulation was repeated after selective denervation of the intrinsic tongue muscles, the increase in velopharyngeal airway volume was significantly attenuated. These findings support our working hypothesis that intrinsic tongue muscles play a critical role in modulating upper airway patency.

sleep apnea; magnetic resonance imaging; velopharynx

METHODS

Animals and surgical procedures. Procedures adhered to the guidelines established by the institutional animal care and use committee at the University of Arizona. Studies were carried out in 16 male Sprague-Dawley rats (240–260 g). Complete image sets (i.e., pre-post distal XII section, with and without electrical stimulation at each of three airway pressures) were obtained in eight animals. Animals were induced with isoflurane (2–2.5%) and maintained via administration of urethane (1.6 g/kg iv). No 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 throughout the experiment. Rectal temperature was maintained at 37°C with a thermistor connected to a servo-controlled heating pad (Harvard Apparatus). The trachea was cannulated (~1 cm) caudal to the larynx, and the animal breathed spontaneously through this catheter. A second tracheostomy was made ~5.0 mm caudal to the vocal folds and a catheter was advanced to a point 1–2 mm rostral to the vocal folds. This catheter was connected in series to a pressure or vacuum source. In all cases the left naris remained open to allow airflow, whereas the right naris was sealed (see Fig. 1).

Nerve preparation. Each animal was studied before and after denervation of the intrinsic tongue muscles. Intrinsic tongue muscle denervation required sectioning of the medial and lateral branches of the hypoglossal nerves bilaterally, ~1.0–1.5 cm distal to the point of bifurcation of the medial and lateral XII branches (see Fig. 2). This eliminated all intrinsic tongue retractor activities (i.e., superior and inferior longitudinal) and the majority of intrinsic tongue protruder activities (i.e., verticalis and transversus muscles) but preserved innervation to the extrinsic protruder (i.e., genioglossus) and retractor (i.e., styloglossus and hyoglossus) muscles (i.e., extrinsic activation only). This method is based on previously published anatomy (27), and we have shown that this technique successfully eliminates intrinsic tongue muscle activities (3, 5).

Experimental protocol. The study also incorporated stimulus and no-stimulus conditions. Thus each animal was studied with and without electrical stimulation applied to the hypoglossal nerves, both before and after intrinsic tongue muscle denervation.

To deliver electrical stimulation, the hypoglossal nerves were first exposed by a ventral approach (13). The main trunk of the XII nerve, i.e., that portion of the nerve located proximal to the bifurcation of the whole hypoglossal nerve into medial and lateral branches (see Fig. 1), was inserted into a nerve-cuff electrode. Each cuff comprised a (3.0 mm) length of PE tubing (1.0 mm ID) cut on the long axis to create the upper airway in humans and rats (10, 19, 20). Our primary objectives were to test the following hypotheses: 1) that VP airway volumes are greater in the hypoglossal intact animal than in the intrinsic denervated preparation and 2) that electrical stimulation of the hypoglossal nerve is more effective in increasing VP airway volume when nerve supply to the intrinsic tongue muscles is preserved. Our findings support both these hypotheses.

THE TONGUE COMPRISES INTRINSIC (origin and insertion within the tongue) and extrinsic (external bony origin and insertion into the tongue base) muscle fibers. There are four intrinsic tongue muscles (verticalis, transversus, superior, and inferior longitudinal) and four extrinsic tongue muscles (genioglossus, hyoglossus, styloglossus, and palatoglossus). Despite evidence of in-series (14) and in-parallel (27) arrangements of intrinsic and extrinsic tongue muscle fibers, there is a widely held belief that extrinsic tongue muscles alter tongue shape (1, 8, 33). Based in large part on this conceptual distinction, research in the area of obstructive sleep apnea has focused exclusively on the extrinsic tongue muscles and the potential for these muscles to alter airway caliber (9, 11, 12, 17, 21, 24, 30, 34, 38).

In recent years our laboratory has focused on the intrinsic tongue muscles and shown that these muscles, which comprise the bulk of the tongue, are coactivated with extrinsic tongue muscles in eupnea, hypercapnia, and hypoxia (3, 5, 6). Our findings raised the possibility that intrinsic tongue muscles, by virtue of their extensive interdigitation with extrinsic tongue muscles, can alter the mechanical properties of the extrinsic tongue muscles and in turn affect pharyngeal airway volume.

Our attention in this study was focused on the velopharyngeal (VP) airway, identified as a highly collapsible segment of

Address for reprint requests and other correspondence: E. F. Bailey, Dept. of Physiology, College of Medicine, The Univ. of Arizona, Tucson, AZ 85721-0093 (e-mail: ebailey@u.arizona.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
a hemispherical vessel that permitted access to the interior concave surface. Two Teflon-coated (0.005-in.) platinum wires with \(0.8 \text{ mm}\) exposed at the tips were secured to the inner surface of the cuff, perpendicular to the long axis. Each nerve was placed into the cuff, which enclosed the nerve. The platinum wires were attached to 28-gauge shielded copper wires connected to a stimulus isolation unit. Stimulation parameters were consistent with those used in previous studies (7). A two-channel stimulator (Grass model S-1188, Quincy, MA) was used in combination with two constant-current stimulus isolation units (Grass, PSIU8). In this manner, any difference in the impedance between the right and left hypoglossal nerve-cuff electrodes could be accommodated via manipulation of the current delivered to each electrode. All other settings on the stimulator were the same for right and left electrodes. Square-wave pulses (0.1 ms) were delivered at 90 Hz to the intact whole hypoglossal nerves to obtain fused contractions (7).

In each animal, stimulus maximum and threshold were first determined before placement into the magnet as follows. First, the tongue was placed behind the upper incisors and then, with the mandible slightly elevated, a low threshold current, defined as the lowest stimulus current to cause visible tongue movement, was delivered to each cuff electrode. The maximum current was defined as the current above which tongue protrusion or retraction was maximal. Threshold and maximum stimulus levels were determined separately for each electrode. A stimulus level one-third to one-half maximum was used for initial testing in the magnet. To ensure comparable stimulation bilaterally, protrusion or retraction was compared separately for right and left electrodes at each stimulus intensity. Prior to imaging, the rat was transferred to a water-heated pad mounted on a 4.5 \(\times\) 60 cm Plexiglas platen. The platen consisted of Plexiglas ear bars that fixed the head and minimized head motion during nerve stimulation. Because the imaging protocol comprised multiple trials, the position of

Fig. 1. Schematic representation of the experimental configuration. Bottom: enlargement of the ventral neck region and depicts the location of the tracheotomy tube, hypopharyngeal catheter, and site of application of positive and negative pressures. Sites for sampling hypopharyngeal (Php) and pharyngeal airway pressures and the approximate location of the cuff electrodes (represented by boxes), proximal to the bifurcation of the XII nerves are also shown.

Fig. 2. Schematic lateral view of the left side of tongue with tongue tip to the left. The hypoglossal nerve is seen to bifurcate near the tongue base and ramify into the tongue body. The lateral division of the hypoglossal nerve (XIIl) has the nerve to the styloglossus (sg) and hyoglossus (hg) muscles branching from it and then continues into the tongue body to divide into dorsal and ventral branches that supply the superior (SL) and inferior longitudinalis (IL) intrinsic muscles of the tongue. The medial division (XIIm) sends several branches to the geniobhyoid (gh), then enters the genioglossus (gg) muscle sending several nerve branches into the belly of the gg, and then courses into the substance of the tongue, continuing to give off small branches to verticalis and transversus intrinsic muscles throughout the tongue length. Arrows indicate the sites of distal XII nerve sections. Approximate location of the nerve cuff electrode on the main XII nerve trunk is indicated at right. Modified and reprinted with permission of authors and publisher (27).
the tongue in the oral cavity and the stimulus intensity were reevaluated before each of the imaging sequences and after the animal was reinserted into the magnet after denervation of the intrinsic tongue muscles.

**Imaging.** An Avance Bruker Biospec MRI instrument (Bruker Instruments, Billerica, MA) was used for all imaging studies. This instrument was equipped with a 40-cm bore, 4.7-T superconducting magnet, an actively shielded gradient coil, and a 72-mm-diameter volume radiofrequency coil. The imaging protocol has been described in detail previously (7). The protocol comprised two imaging sequences: gradient echo fast imaging (GEFI), and spin echo, rapid acquisition with relaxation enhancement (RARE) imaging.

GEFI. These images were used to visualize tongue movements in situ, to optimize stimulus current levels, and to check the viability of the nerve preparation before and after each set of RARE images was obtained (see below). The settings used for GEFI imaging were echo time, 6.8 ms; relaxation time (TR), 20 ms; flip angle, 30°; number of excitations, 1; field of view, 8 × 8 cm; matrix size, 128 × 128 lines. This GEFI protocol yielded a temporal sequence of 12 images, with each image requiring 4.4 s. Nerve stimulation was delivered during a discrete 16-s interval that was bracketed by two 16-s intervals without stimulation. This provided four serial images without stimulation, four with stimulation, and four after stimulation.

Stimulus-gated RARE. This protocol was used to acquire high-resolution axial and sagittal images of the velopharyngeal airway with and without stimulation applied to the hypoglossal nerves. The RARE technique minimizes artifacts that arise as a result of inhomogeneities in the magnetic field. Air and tissue have different magnetic susceptibilities, and, as a result, the magnetic field is distorted at the air-tissue interface (7).

Because stimulating the muscle continuously could result in fatigue, the imaging protocol involved acquiring data during short, repetitive trains of motor nerve stimulation, with each stimulus train separated by a recovery period. On the basis of our previous work, we applied a 1.7-s stimulus train every 5 s (7). Images were acquired during the stimulus period by triggering the acquisition software with the stimulus output pulse. The trigger was set so that image capture did not begin until 50 ms after the onset of stimulation to allow time for the tongue muscles to shorten fully. To obtain images without stimulation, the electrode wires were disconnected from the stimulation isolation unit and the protocol was repeated.

Settings for the RARE imaging protocol were as follows: excitation time, 6.8 ms; TR, 5 s; field of view, 3.2 × 3.2 cm; matrix size, 128 × 128 lines. These settings resulted in the collection of 8 of the 128 lines of data during each TR period [i.e., an excitation train length (ETL)] of 8. We used two excitation periods, yielding a total imaging time of 160 s (128 lines × 2 excitations × 5 s TR/ETL = 8).

**Image analysis.** We defined the nasal VP airway as the airway region on the dorsal side of the soft palate that extends from the junction of the hard and soft palate to the tip of the soft palate inferiorly. The oral VP airway was defined as the portion of the airway on the ventral side of the soft palate encompassing the oral airway lumen between the tongue and the soft palate.

The perimeters of the nasal and oral VP lumens within each axial slice were traced, using image software (Scion Image) to obtain measures of cross-sectional area (CSA) at each slice location. Total VP airway volume (oral + nasal) was calculated as the sum of the individual CSAs of the analyzed slices. As previously (7), to register the axial slices in a consistent manner across experiments we placed an “alignment slice” at the notch between the olfactory bulb and the cerebral hemisphere. The location of the notch approximates the junction of the hard and soft palates (see Fig. 3A). Once the alignment slice was identified, we measured 1.0-mm slices starting 2 mm distal to the alignment slice and extending throughout the length of the nasal and oral VP airways. We analyzed a total of seven slices for the oral VP airway and seven slices through the nasal VP airway. The same number of slices was obtained for oral and nasal VP airways in all animals. Because the focus of our work is on the VP, airway regions caudal to the tip of the soft palate and rostral to the junction of the hard and soft palates are not included in our analysis.

**Inferential statistical analysis.** Differences in the average VP airway CSAs were assessed initially via a single (3 × 2 × 2 × 2 × 7) within-subjects repeated-measures ANOVA testing main effects (and interactions) for pressure (+5.0, atmospheric, −5.0 cmH2O), airway (nasal vs. oral), nerve (intact vs. denervated), stimulus (stimulus vs. no stimulus) and slice location (1–7). Significant main effects were found for the variables nerve, pressure, and slice location. Significant two-way interactions were found for airway region × pressure, airway region × nerve, nerve × pressure, nerve × slice, nerve × stimulus, and slice × pressure. When a significant interaction was found it was tested where appropriate and physiologically relevant, by using post hoc adjacent level contrasts with significance levels adjusted according to the Bonferroni procedure (P < 0.05).

**RESULTS**

Figure 3 shows midsagittal and axial MRIs of the rat in the supine position at atmospheric pressure. The axial images in Fig. 3A show the nasal and oral VP airways in the spontaneously breathing animal in the no-stimulus condition. Figure 3B shows the same animal after nerve supply to the intrinsic tongue muscles was removed. Intrinsic denervation reduces airway area in the rostral oral VP, and there is narrowing in the more caudal region of the nasal VP that can be observed by comparing corresponding sagittal and axial images in Fig. 3.

Figure 4 shows midsagittal and axial MRIs at an airway pressure of positive 5 cmH2O, obtained from the same animal depicted in Fig. 3. Figure 4A shows the nasal and oral airways in the spontaneously breathing animal in the no stimulus condition. Both the oral and nasal VP airways are open and the CSA of the VP airway is greater under the positive pressure condition (i.e., compare sagittal views Fig. 3A and 4A and axial slices). Figure 4B shows the same animal after denervation of the intrinsic tongue muscles. As seen in the sagittal view and accompanying axial images, denervation of the intrinsic tongue muscles reduces the airway opening in the rostral and caudal regions of the oral VP. The nasal VP CSA remains unchanged and is patent along its length.

Figure 5 shows midsagittal and axial MRIs at an airway pressure of −5.0 cmH2O. All images were obtained from the same animal depicted in Figs. 3 and 4. The sagittal view in Fig. 5A shows closure of the oral airway along its length, and this observation is confirmed in the accompanying axial slices. In contrast, the nasal airway is patent throughout its length. Figure 5B shows the VP airway after bilateral section of the nerve supply to the intrinsic tongue muscles. In this case, denervation does not appear to have any significant effect on either the nasal or oral airway CSA.

Mean (SD) oral and nasal VP airway CSA at each slice location and at each airway pressure (+5 cmH2O, atmospheric and −5 cmH2O) before and after intrinsic tongue muscle denervation are shown in Fig. 6A. Denervation of the intrinsic tongue muscles brought about significant reductions in oral CSA at positive and atmospheric airway pressures (P < 0.003) but did not affect airway CSA at −5 cmH2O VP pressure. In contrast, intrinsic denervation did not affect the CSA of the nasal airway at any slice location or airway pressure (P > 0.129).

Figure 6B depicts the effects of hypoglossal nerve stimulation on the CSA of oral and nasal VP airways before and after intrinsic tongue muscle denervation. Specifically, stimulation of both intrinsic and extrinsic tongue muscles was associated
with significantly larger oral VP airway CSA at both positive and atmospheric airway pressures relative to the intrinsic denervated preparation ($P < 0.003$). The same trend was evident at the $-5 \text{ cmH}_2\text{O}$ airway pressure although the magnitude of the difference between the XII intact and intrinsic denervation preparations was significant for airway slice locations 7–9 only ($P < 0.003$). Importantly, electrical stimulation with all extrinsic and intrinsic tongue muscles intact resulted in larger average nasal VP airway CSA compared with the intrinsic tongue muscle denervation condition at atmospheric and negative airway pressure conditions and at almost all slice locations.

Figure 7 presents average (SD) total (oral + nasal) VP airway volumes for the group. The top graph depicts the total airway volume at the three test pressures, both before and after denervation of the intrinsic tongue muscles. The bottom graph depicts the influence of XII nerve stimulation on total airway volume at each of the three test pressures both before and after denervation of the intrinsic tongue muscles. In the intact preparation, total VP airway volume was dependent on airway pressure (top, $P < 0.001$). Intrinsic denervation did not exert any significant effects on VP volume at $+5.0 \text{ airway pressure}$ ($P < 0.8$) but significantly reduced VP volume at atmospheric pressure ($P < 0.03$) and increased volume at a pressure of $-5.0 \text{ cmH}_2\text{O}$ ($P < 0.043$). These effects of intrinsic muscle denervation were magnified when denervation was paired with electrical stimulation of the hypoglossal nerves.

**DISCUSSION**

**Summary.** These experiments demonstrate for the first time that intrinsic tongue muscles play a crucial role in determining...
total VP airway volume and CSA at pharyngeal pressures ranging from −5 to 5 cmH₂O. We also show that electrical stimulation of the hypoglossal nerves is significantly more effective in dilating the VP airway when the intrinsic and extrinsic tongue muscles are coactivated.

**Methodological issues.** Under the present protocol, animals were anesthetized with intravenous urethane. We have previously demonstrated intrinsic and extrinsic tongue muscle activities during spontaneous breathing in rats using the same anesthetic protocol as used herein (3, 5). Urethane is an appropriate anesthetic in this instance because it has a minimal depressant effect on respiratory function and does not require assisted ventilation (23).

Importantly, we have also shown that it is possible to selectively denervate intrinsic tongue muscles while leaving extrinsic activities intact under the same condition (3). These previously published observations and the denervation protocol form the foundation for the present imaging study.

We focused exclusively on the oral and nasal segments of the VP airways for two reasons. First, it this region of the upper airway that is considered most “collapsible” in patients diagnosed with obstructive sleep apnea (19, 20, 31). Second, studies in rat demonstrate marked improvements in VP airway geometry and flow mechanics with electrical stimulation applied to the whole hypoglossal nerve (7, 10).

Relative to the L-shaped human upper airway, the supine rat airway is more rectilinear. Nevertheless, the rat is an appropriate model for studies of this type because there is considerable homology among the rodent and human pharyngeal airway in terms of anatomy, innervation, and mechanical actions of the tongue muscles (5, 27–29). Despite the comparative rectilinearity of the rodent airway, certain sites along the airway may have been somewhat oblique to the orientation of the airway. We made no correction for such changes in airway orientation because each animal served as its own control. Thus, although a given axial slice may not be precisely ortho-
onal to the airway centerline, the angle of orientation would be the same under all conditions. Thus any differences in airway volume due to orientation artifact would be constant under all conditions in a given slice and hence would not impact the calculated changes in volume consequent to alterations in pressure or nerve stimulation.

There are several nontrivial technical challenges associated with this preparation that are worthy of comment. In particular, the need to maintain the mucous membranes in a healthy condition is of paramount importance in a protocol of this type and duration. Attempts to obtain a “dry” airway, i.e., one that is void of secretions, either via administration of atropine, delivery of dry tank air, or repeated suctioning, proved deleterious to the viability of the preparation. Accordingly, we maintained a warmed and humidified upper airway and in so doing minimized accumulation of secretions and reduced surface adhesion forces that might otherwise have brought about airway closure.

In these studies we examined the influence of tongue muscle contraction on VP airway CSA under conditions of steady-state flow. In contrast, previous studies have used sealed, isolated upper airway preparations to evaluate changes in pharyngeal compliance with upper airway muscle stimulation (4, 15, 22). The reason that we studied the system under dynamic flow conditions is fourfold. First, our pilot studies revealed that the sealed upper airway preparation suffers from a buildup of secretions that make MRI difficult if not impossible, because fluid buildup is associated with image artifacts at the fluid-air interface. Second, because the protocol required that the animal remain positioned in the scanner for several minutes, it was not possible to repeatedly remove and reinsert the animal to clear the airway of secretions and maintain the geometric configu-

---

Fig. 5. A: representative sagittal and axial MR images in a hypoglossal intact at −5.0 cmH₂O pharyngeal airway pressure. Details as above. B: the same preparation after intrinsic tongue muscle denervation preparation.
ration of landmarks that are crucial for reliable imaging. Third, we are interested in the effects of tongue muscle activity on VP CSA at different dynamic pressures and did not attempt to measure compliance in these experiments. Fourth, and perhaps most importantly, the deformation of a mechanical structure in response to a particular steady state does not depend on how that load is applied. That is, a steady-state load can be achieved with a constant flow source, as long as the flow is steady. The flow may, however, alter the distribution of the loading. Thus, if there is a significant pressure gradient, then the pressure will be different at different points in the channel and shear stresses will act in parallel to the channel surface. However, the expected variations in pressure along the segment of the pharynx that we analyzed are expected to be small relative to the magnitude of the pressures that we applied.

We used modified GEFIs at the start and end of each pressure condition. These images enabled us to visualize tongue movements in situ and thus ensure the adequacy, efficacy, and consistency of stimulation-induced tongue movements throughout the imaging protocol. Our success in maintaining a viable electrical contact with the nerve is attributed to the design of the cuff electrode. Previously, it was necessary to section the XII nerves and pull the peripheral nerve end through the cuff (7). In the present study, however, we revised the electrode design to preserve the integrity of the XII nerve and its blood supply. The new cuff proved to be a more effective and stable mode of current delivery that eliminated the need to adjust stimulation parameters during the imaging protocol.

In this experiment we stimulated the hypoglossal nerve at a single stimulation frequency of 90 Hz. The decision to stimulate at this frequency was based on previously published observations in the rat isolated airway preparation that demonstrated fusion at $90 \text{ Hz}$ as well as significant increases in maximal inspiratory flow and more negative collapsing pressures for frequencies $60 \text{ Hz}$ or higher (10).

Recent anatomical work in the rat indicates that section of the medial and lateral XII branches distal to the point of bifurcation may fail to completely eliminate innervation of intrinsic tongue muscles that participate in tongue protrusion and retraction (27, 32). Thus it is possible that some intrinsic activities persisted after denervation. However, if this is the case, the present data set underestimates the contribution of the intrinsic tongue muscles to the observed increases in total VP volume and CSA.

We measured regional airway CSA and total VP airway volume at three test pressures. Atmospheric pressure served as the control condition, and this was compared with $+5.0 \text{ cmH}_2\text{O}$ and $-5.0 \text{ cmH}_2\text{O}$ pressure applications. A positive pressure of $+5 \text{ cmH}_2\text{O}$ was chosen because it approximates a level of continuous positive airway pressure used in the treatment of patients with obstructive sleep apnea. Similarly, a negative pressure of $-5 \text{ cmH}_2\text{O}$ was chosen. The expected variations in pressure along the segment of the pharynx that we analyzed are expected to be small relative to the magnitude of the pressures that we applied.
The focus of the present study was the VP airway and more specifically what effect intrinsic tongue muscle denervation had on VP CSA and total VP airway volume. Although the present findings certainly indicate that intrinsic tongue muscles work synergistically with extrinsic tongue muscles to preserve VP airway opening, a more in-depth analysis of the mechanisms underlying the muscle synergy lies beyond the scope of the present work.

The authors thank the National Institutes of Health (NIH) of the United States Public Health Service for supporting the studies (NIH grants DC-07597 and HL-56876).
REFERENCES


