Mechanical effects of genioglossus muscle stimulation on the pharyngeal airway by MRI in cats

Michael J. Brennick a,∗, Warren B. Gefter b,1, Susan S. Margulies c,2

a Center for Sleep and Respiratory Neurobiology, Department of Medicine, University of Pennsylvania, 991 Maloney Building, 3600 Spruce Street, Philadelphia, PA 19104, United States
b Radiology Department, University of Pennsylvania, Philadelphia, PA 19104, United States
c Biomedical Engineering Department, University of Pennsylvania, Philadelphia, PA 19104, United States

Accepted 22 August 2006

Abstract

To examine the regional mechanical effects of selective genioglossus muscle activation on pharyngeal airway size and function, magnetic resonance images of the pharyngeal airway were obtained in five paralyzed, anesthetized cats over a range of positive and negative pressures in an isolated, sealed upper airway. When all results across pressure levels and pharyngeal regions were analyzed, genioglossus stimulation significantly increased the cross-sectional area (CSA) of the nasopharyngeal airway. Within specific regions, stimulation tended toward significantly increasing cross-sectional airway area in the mid-nasopharynx. Despite its dilating effect, genioglossus muscle stimulation did not alter compliance in the nasopharyngeal airway, as evidenced by the similar slopes of the pressure versus cross-sectional area relationships with and without stimulation. Finally, airway shape in the mid pharynx became more circular with either increased airway pressure or genioglossus stimulation. The results indicate that selective stimulation of the genioglossus muscle dilates the nasopharynx and provide evidence that stimulation of the genioglossus alone does not alter airway compliance.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Airways, Upper, Muscles; Muscles, Genioglossus; Sleep, Obstructive apnea; Magnetic resonance imaging

1. Introduction

Airway obstruction in patients with obstructive sleep apnea (OSA) is believed to arise from neuromuscular factors related to the reduction in upper airway muscle activity during sleep and predisposing anatomical factors such as decreased pharyngeal airway size and increased pharyngeal airway compliance (Schwab et al., 2005). The most commonly used treatments for patients with OSA, including nasal continuous positive airway pressure, oral mandibular advancement devices, and pharyngeal airway surgery, are not efficacious in all patients (Rodenstein, 1992; Launois et al., 1993; Isono et al., 1999a; Hicklin et al., 2000; Senior et al., 2000; Weaver, 2002; Barnes et al., 2004). Several studies have demonstrated the feasibility of electrical stimulation of tongue muscles as a possible treatment for patients with OSA (Schwartz et al., 1996; Oliven et al., 2001, 2003; Schwartz et al., 2001). In those studies, stimulation of the lingual muscles increased airflow and reduced the number of obstructions during sleep. However, progress and development of muscle stimulation methods for OSA therapy or indeed any novel pharmacological therapies (Veasey, 2001) will benefit and rely on more complete knowledge as to which muscles or muscle combinations can reduce collapsibility either through airway dilation or reduced compliance of the pharyngeal airway during sleep.

Previous investigators have examined the mechanical effects of tongue muscle activation on the pharyngeal airway using a variety of techniques with somewhat conflicting results. The relative contribution of muscle groups innervated by the hypoglossus nerve (Getty, 1975; Williams, 1995) have been studied by selective nerve stimulation whereby, “whole nerve” stimulation of both medial and lateral branches has been used to stimulate the genioglossus geniohyoid, styloglossus, hyoglossus and
intrinsic tongue muscles, while medial branch stimulation was used to selectively stimulate only the genioglossus, geniohyoid and intrinsic tongue muscles (Fregosi and Fuller, 1997; Fuller et al., 1998; Kuna and Brennick, 2002). Using a Starling resistor model of flow in the upper airway, Fregosi and Fuller (1997) and Fuller et al. (1998) found that ‘whole’ hypoglossal nerve stimulation decreased critical pressure ($P_{crit}$, collapsing pressure of the flow limiting segment) but had little effect on maximal inspiratory airflow ($V_{Imax}$), while stimulation of just the medial branch of the hypoglossal nerve increased $V_{Imax}$ but had a minimal effect on $P_{crit}$. These results were interpreted to suggest that selective stimulation of the medial hypoglossus merely dilates the airway, while stimulation of both medial and lateral hypoglossus branches may reduce airway collapsibility by dilation and by altering pharyngeal airway tissue characteristics (reduced $P_{crit}$) (Eisele et al., 1995; Fregosi and Fuller, 1997; Fuller et al., 1999; Kuna and Brennick, 2002). In contrast to those results, Kuna and Brennick (Kuna and Brennick, 2002) measured static compliance (using a fiberoptic method in the isolated airways in decerebrate cats) and found that pharyngeal compliance, as the slope of airway pressure versus cross-sectional area ($CSA$) relationship, was reduced during stimulation of either the whole hypoglossal nerve or just its medial branch.

Pharyngeal compliance was also measured in a fiberoptic study in anesthetized paralyzed patients with OSA by Isono et al. (1999b), who reported that transmucosal stimulation of tongue muscles of the oropharyngeal segment near the epiglottis decreased pharyngeal airway compliance. Finally, in a comparison study of genioglossus muscle stimulation using fine wire intramuscular electrodes or unilateral stimulation of the medial branch of the hypoglossal nerve in OSA patients during sleep, Oliven et al. (2003) reported that both intramuscular and nerve stimulation methods increased airflow, reduced $P_{crit}$, and reduced apnea severity. They noted however, that with either method of stimulation, the increase in maximal inspiratory airflow ($V_{Imax}$) occurred without a change in upstream resistance and suggested that the lowered $P_{crit}$ in these patients occurred without a reduction in compliance.

The purpose of this study was to examine the regional mechanical effects of selective genioglossus muscle activation on pharyngeal airway size and function in paralyzed, anesthetized cats. We used magnetic resonance imaging (MRI) with and without genioglossal stimulation over a range of applied positive and negative pressures in an isolated, sealed upper airway and measured airway cross-sectional area (CSA) versus airway at multiple rostral to caudal regions of the pharynx. We reasoned that a reduction in the slope of the resulting airway pressure versus CSA relationships with genioglossus stimulation would indicate that stimulation decreased airway compliance, whereas a parallel shift would indicate that airway compliance was unchanged. If there were no change in compliance but an increase in CSA across pressure levels then the upward shift of the pressure versus CSA curve would be reflected in a reduced closing pressure ($P_{close}$ or pressure at 0 CSA). The ratio of the lateral to anteroposterior (AP) airway diameters was also examined in each region to determine what shape changes, if any, occurred due to genioglossus stimulation and the different levels of pressure.

2. Methods

2.1. Surgical preparation

The methods were approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC). Five cats of either sex, weighing 3.2 ± 0.2 kg (mean ± S.E.M., throughout text) were pre-anesthetized using intramuscular Ketamine (15 mg/kg) with intramuscular diazepam (2 mg/kg) for muscle relaxation and atropine (0.05 mg/kg) for reduction of secretions and to aid anesthetic induction. Within 15–30 min of anesthetic induction, isoflurane vapor (1.5–2.0% by volume in pure O$_2$) was administered via a fitted facemask. No surgery was performed unless reflex response to strong pressure on the hind paw was absent.

Preparation of the isolated, sealed upper airway was performed as previously described (Brennick et al., 1998). Briefly, the cervical trachea was exposed from the cricoid cartilage to the sternal notch and a 7–8 mm section of trachea approximately 2 cm caudal to the cricoid cartilage was replaced with a custom designed tracheotomy tube of equal length with separate openings to the caudal and rostral trachea. The animals were connected to a mechanical ventilator (Harvard Respirator, Millis, MA) with the isoflurane vaporizer (1.5–2.0% by volume in pure O$_2$) placed in-line. Tidal volume was 50–60 ml, and respiratory rate was adjusted to achieve an end-tidal $P_{CO_2}$ (Datex Normocap 100, Tewksbury, MA) between 28 and 32 Torr. During the entire protocol, end tidal $CO_2$ ($P_{CO_2}$), arterial oxygen saturation and heart rate were monitored (VetOx 4000, SDI, Waukesha, WI). The esophagus was ligated below the larynx. Through the rostral tracheal cannula, a smaller tube (2 mm o.d.) was advanced through the vocal cords to just below the base of the epiglottis to prevent vocal cord adduction and allow equalization of pressure above and below the larynx. A femoral venous catheter (Abbocath® T-18G × 2”) was inserted for post-surgical administration of neuromuscular blockade drug gallamine triethiodide (30 mg/kg).

2.2. Genioglossus electrical stimulation

A single bipolar electrode pair (Model E2, 10 mm platinum subdermal needle electrodes, Grass Instrument, Quincy, MA) was implanted in the right and left genioglossus muscle using an intra-oral approach, 2–5 mm posterior to the frenulum of the tongue in the region of the oral sulcus. The exposed needle tips, having passed through the genioglossus muscle, were anchored with cyanoacrylate glue onto a 2 mm$^3$ cork plug. The PVC coated electrical leads of the needle electrodes were twisted to reduce electromagnetic interference in the MRI magnet and placed between the upper and lower lips before sealing the mouth. Electrical muscle stimulation was delivered using a Grass S88 stimulator (Astro-med, Quincy) at a frequency of 50 pulses per second and 0.5 ms pulse duration for a given current intensity using a constant current stimulus photo-isolation...
In all cases the airway was at atmospheric (0 cm H₂O) pressure, and the cat was in the supine position. In panel (A), there was no applied stimulation; in panels (B and C), stimulation at 50 Hz, 0.5 ms duration was 1.5 mA (B) and 3.0 mA (C). Levels of current intensity higher than 3.0 mA (not shown) did not further increase the size of the pharyngeal nasopharynx. See Fig. 4 for annotation details of pharyngeal axial cross-sections.

Correct placement of the electrodes was evaluated at three stages during the experiment. First, following electrode implantation, the genioglossus was stimulated and anterior tongue protrusion was directly observed. If the muscle stimulation did not produce symmetrical protrusion, the preparation was examined and, if needed, the electrodes were repositioned. These “bench” observations and adjustments were performed prior to sealing the isolated upper airway and resulted in nearly symmetrical contractions in all cats. Secondly, tongue displacement (in anterior ventral direction) expected to some degree during genioglossus stimulation, was checked in the initial MRI acquisitions from on-line sagittal observations at the MRI console. Thirdly, post-experimental necropsy on each cat was performed to confirm that the electrodes remained in their initial implanted position.

2.3. Pressure control in isolated upper airway

Following placement of the genioglossus electrodes, the oral cavity was closed and secured with the anterior edge of the tongue positioned at the lower incisors. The airway was sealed around the mouth using 1-0 Vicryl® suture (Ethicon, Somerville, NJ) and cyanoacrylate gel glue. One naris was plugged with cotton and sealed with the glue. Airway pressure was measured by a non-magnetic pressure transducer (SPC350MR, Millar Instruments, Houston) connected to a cannula placed in the second naris that was then also sealed. This signal was amplified through a low noise amplifier (PM1000, CWE, Inc., Ardmore, PA), visualized on a Tektronix oscilloscope (Type D54, Beaverton, OR), and recorded on a Gould recorder (ES1000, Cleveland).

Positive or negative (vacuum) pressure was delivered to the isolated, sealed upper airway by thick walled tubing (1/4 in. i.d. × 1/8 in. wall Tygon®, Norton Plastics, Akron, OH). Pressure control was achieved by adjusting a fine needle valve connected to a low-flow bleed port on the pressure delivery line. Pressure levels were held constant, in spite of any possible slight leaks in the airway segment, since the system was designed to control pressure and not volume. Once a steady state was achieved following surgery, MR images of the pharyngeal airway were acquired with and without genioglossus muscle stimulation at the following airway pressures: −5.0, −2.5, 0, 2.5, and 5.0 cm H₂O. The mean airway pressures measured during image acquisition in the five cats were: −5.02 ± 0.06, −2.22 ± 0.11, 0.0 ± 0.0, 2.34 ± 0.08, and 4.92 ± 0.22 cm H₂O. Imaging was performed in the following “pressure-level order”: 0, ±5.0, −5.0, +2.5, and −2.5 cm H₂O.

2.4. MRI acquisition, registration and mensuration

MR imaging was performed in a 1.9 T, 40 cm diameter magnet interfaced to a General Electric Signa, version 4.7× computer and console. A Spin-echo MRI sequence (repetition time, TR = 2000 ms, echo time TE = 20 ms, with one excitation per view, over a 10 cm × 10 cm axial field of view, on a 256 × 128 pixel grid) was used to acquire images of pharynx (20 axial slices, thickness 3 mm) using a quadrature radio-frequency volume coil specially designed to fit snugly over the cat’s head and tuned to the 200 MHz resonant proton frequency. The images were interpolated at the Signa 4.7× console to a 256 × 256 pixel grid with in-plane resolution of 0.391 mm/pixel. A limited number of sagittal images were also acquired on 12 cm × 12 cm field of view. The MRI sequence utilized a stimulus-gated protocol that was triggered by the stimulator (Fig. 2). The stimulus pulses were applied in a repeated train, on for 700 ms and off for 1300 ms. Muscle stimulation was applied 200 ms prior to stimulus-on image acquisition so that images were obtained when the muscle is in a steady state of contraction. The stimulus-on and stimulus-off times were chosen to approximate the inspiratory and expiratory times and duty cycle (muscle active time/respiratory cycle time) for a typical awake cat (Bonora et al., 1985). At the end of the experiments, euthanasia was performed using a pentobarbital overdose (300 mg/kg, i.v.).

Axial images (3 mm thick) of the pharynx were acquired at each experimental condition. Six axial images were selected that sampled the pharyngeal airway over the extent of the soft palate from the junction of the hard and soft palate to the distal edge of the soft palate (see Fig. 3). In order to compare images from all five cats, fixed bony anatomical landmarks were used to match the axial regions from one cat to another. The most rostral image
Fig. 2. Schematic representation of the spin-echo gated MRI protocol. Muscle stimulation (initiated by the Grass® stimulator) began 200 ms before MR image acquisition was triggered. Pulse encoding (over pharyngeal tissue volume) with an echo time (TE) of 20 ms occurred during the following 500 ms (shaded: striped section) while muscle stimulation continued. Tissue relaxation without stimulation followed for 1300 ms to provide an overall repetition time (TR) of 2000 ms. This sequence was repeated 128 times to acquire a complete image matrix and the entire 128 cycle gated sequence was repeated for 4 averages (not shown) to increase signal to noise in each image series. Unstimulated images were acquired in separate series using the same protocol except without application of stimulus current. The gated spin-echo MRI protocol provided excellent proton density image contrast using repeatable short bursts of muscle stimulation.

was at the eye orbit. Five succeeding images (every other 3 mm slice of the interleaved sets) completed the set for analysis with the most caudal (sixth) image at the level of the tympanic bulla.

Airway CSA and the anteroposterior (AP) and lateral diameters were measured in the nasopharyngeal airway in all images. These measures in the oropharyngeal airways were obtained in those regions where the oropharyngeal airway was patent in all cats. As previously described, a thresholding method was employed to obtain these measurements (Brennick et al., 1998, 2001). Briefly, for the images displayed in 256-bit resolution of grey values, a threshold value was determined equal to the pixel value of the black space (airways) plus a correction for noise (noise estimated as the standard deviation of a homogeneous or dark region of the image (McGibney and Smith, 1993). The threshold value for each image series was then used to differentiate the airways from the surrounding tissues by NIH-Image (v. 1.61/ppc, US National Institutes of Health http://www.rsb.info.gov/nih-image). The NIH-Image program also computed the AP and lateral diameters using a built in algorithm for an ellipse best fitted to the airway area. Airway ‘elliptical ratio’, defined as (lateral diameter)/(AP diameter), was used to determine the effects of stimulation on airway shape. An elliptical ratio of 1.0 indicates a circular shape and a value greater than 1.0 indicates that the airway is elliptical with greater elongation in the lateral diameter.

2.5. Statistical analysis

The analysis examined the effect of stimulation versus no stimulation on CSA measurements, and whether the effect of stimulation was modified by pressure (five nominal levels: +5, +2.5, 0, −2.5 and −5 cm H2O) and region (six regions). In short, was the effect of stimulation (if present) different at various pressure levels and/or different regions? The ANOVA General Linear Model (SAS Institute, Cary) computed a separate intercept for the regression of CSA on pressure level for each region, and modeled a linear relationship between pressure and CSA (Littell et al., 1996). The main effects of pressure, region and stimulation and higher order interaction terms, i.e., pressure × region, stimulation × pressure, and stimulation × region, were tested against the dependent variable CSA (significance assumed for $p<0.05$). Quantitative analysis by linear regression over the airway pressure versus CSA relationship in each region was used to determine the estimated $P_{\text{close}}$ i.e., the $Y$ intercept or pressure value where CSA = 0. Paired $t$-test was used to determine if stimulation caused a significant change in $P_{\text{close}}$ for a given region. For the regression analyses, one cat was omitted since complete pressure data for that animal was not available (although statistical methods allowed the cat with missing data points to be included in the ANOVA model). The effects of pressure, stimulation, and pressure × stimulation on the elliptical ratio were evaluated by two-way ANOVA within each region. Differences across regions (or within regions for shape analysis) were evaluated using raw $t$-values and Bonferroni or Student–Newman–Keul’s (SNK) adjustment ($p<0.05$) where appropriate (Winer et al., 1991).

3. Results

Fig. 3 (left image) shows the pharyngeal regions that were analyzed on the axial images. Pharyngeal enlargement dur-
ing stimulation (Fig. 3, right image) is evident in the mid and caudal pharyngeal regions. The circular inset in the right panel shows the location of the stimulating electrodes, where the image distortion is a susceptibility artifact that was due to the great difference in T1 relaxation time between the metal electrodes and surrounding tongue muscle tissue. In most animals, this artifact was well out of the range of the pharyngeal airway space. In some of the more axial rostral images, airway boundaries were missing because of susceptibility artifact between the airway and the bones of the sphenoid sinus. If the airway could not be isolated using the threshold values, a boundary was inscribed manually to connect the established edges along the curvature of the airway.

The effects of airway pressure on nasopharyngeal CSA (region 5) in a representative cat with and without genioglossus stimulation are shown in Fig. 4. Relative to the state of the airway at atmospheric pressure, nasopharyngeal airway size progressively decreased with decreasing airway pressure and progressively increased with increasing airway pressure. In this example (Fig. 4) positive airway pressure opened the oropharyngeal airspace that was closed at atmospheric and negative pressures. We found that in all cats, the oropharynx in the most rostral region (region 6) was patent across both positive and negative pressures but that stimulation had no significant effect on oropharyngeal CSA ($p = 0.768$). At the mid-pharyngeal level (regions 4 and 5) the oropharynx was patent at $-2.2 \text{ cm } H_2O$ in only four of five cats and remained closed during stimulation in three of five cats. The oropharynx was, predominately, closed in all animals under all pressures in the more rostral regions (regions 1, 2 and 3). Thus, because patency in the oropharynx was so variable across cats at any given airway pressure and region, it was not possible generate oropharyngeal pressure–area relationships that would allow a reasonable comparison of oropharyngeal CSA with regard to pressure or stimulation.

Results by ANOVA analysis showed that there was a statistically significant overall main effect of pressure ($p < 0.0001$) indicating that CSA values, both stimulated and unstimulated, were dependent upon pressure level. When results from all cats were considered, similar direct relationships between intraluminal pressure and CSA were observed at all nasopharyngeal regions (Fig. 5). Taking into account differences among pressure levels and between stimulated and unstimulated conditions; CSA values were statistically different among the six nasopharyngeal regions ($p < 0.0001$). Post hoc testing of mean CSA in regions showed the following specific differences: region 1 < region 4 ($p < 0.0003$), region 1 < region 5 ($p < 0.034$), while region 2 < region 4 was nearly significant ($p < 0.061$). It was also found that the higher order interaction pressure × region had a significant effect on CSA ($p < 0.0001$) indicating that the effect of pressure on CSA was dependent on region, i.e., the slopes of the pressure–area relationships (airway compliance) differed across pharyngeal regions. Post hoc testing on the data set for only the unstimulated condition showed that airway compliance in regions 4–6 was significantly greater than that in region 1 ($p < 0.05$). However, among nasopharyngeal regions 2–6 there were no significant differences in airway compliance.

Analysis of the data from all cats revealed that stimulation increased nasopharyngeal CSA at atmospheric and subatmospheric airway pressures. Taking into account differences in pressure levels and pharyngeal regions, there was a statistically significant effect of genioglossus stimulation on CSA ($p < 0.031$). However, the interaction term, stimulation × pharyngeal region was not significant ($p = 0.872$), indicating that the effect of stimulation on CSA did not depend on which region was being examined. Post hoc testing on the effect of stimulation at specific pharyngeal regions gave equivocal results. Although the unadjusted $p$-value for the effect of CSA of stimulated versus unstimulated conditions at regions 3 and 4 was significant ($p = 0.016$, both regions), this effect did not reach significance after Bonferroni adjustment (significance predicted at $p < 0.007$). Similarly, stimulated versus unstimulated CSA at region 2 also showed a trend towards significance (unadjusted $p = 0.084$), although not below required adjusted $p$-value ($p < 0.007$). The overall ANOVA results did not support that the effect of stimulation was modified by: pressure (stimulation × pressure, $p = 0.862$) or the joint effects of pressure and pharyngeal region (stimulation × pressure × pharyngeal region, $p = 0.999$). Thus, although stimulation was significant in increasing CSA overall, approaching significance at pharyngeal regions 2, 3 and 4, the lack of a significant stimulation × pressure term indicated that stimulation did not alter the CSA versus pressure relationship, i.e., stimulation did not alter pharyngeal airway compliance.

Table 1 shows results of the linear regression analysis performed over each nasopharyngeal region showing the mean $P_{close}$, linear regression $R^2$ value, and the $t$-test with unadjusted $p$-value, comparing no stimulation to stimulated $P_{close}$. The changes in $P_{close}$ for regions 1–6 respectively, were: $-0.59$, $-5.1$, $-0.88$, $-1.29$, $-1.91$, and $+0.26 \text{ cm } H_2O$ indicating that $P_{close}$ was lowered in all regions except the most caudal region 6, during stimulation. Because the unadjusted $p$-values for the effect of stimulation in a specific region were not less than the Bonferroni predicted $p$-value to assume significance (0.007) these data suggest a trend but not significant changes. Nonetheless, the average change in $P_{close}$ across regions was $-1.67 \pm 0.72 \text{ cm } H_2O$.

The elliptical ratio measurements at each static pressure level, with and without stimulation are plotted in Fig. 6. Statistical analysis revealed no significant shape related changes due to pressure or stimulation in nasopharyngeal regions 1 and 6. In pharyngeal regions 2–5, the results showed that, taking into account changes due to stimulation, there was a significant effect of pressure on the nasopharyngeal elliptical ratio such that increased pressure caused a decrease in elliptical ratio, i.e., the airway became more circular (regions 2–4, $p < 0.001$, region 5, $p < 0.014$). The results also showed that when changes due to pressure were taken into account, genioglossus stimulation significantly reduced the elliptical ratio in regions 2–5 ($p$-value < 0.05). There was no significant interaction between pressure and stimulation on elliptical ratio, indicating that the effect of stimulation was not dependent on pressure level.
Fig. 4. Shown are axial images from region 5 of the pharyngeal airway without (left column images: A, C, E, G and I) and with (right column images: B, D, F, H and J) stimulation at the five pressure levels (top to bottom rows: +5.3, +2.4, 0, −2.0 and 4.9 cm H₂O) in a representative cat. TN = tongue. Other abbreviations as in Fig. 3.
Fig. 5. Mean ± S.E. cross-sectional nasopharyngeal airway area (CSA) vs. mean airway pressure for six regions (NP1–NP6) with (○) and without (●) genioglossus stimulation. See text for description of significant effects in specific regions.

Table 1
Effect of genioglossus stimulation on closing pressures in rostral to caudal nasopharyngeal regions

<table>
<thead>
<tr>
<th>Region</th>
<th>P_{close} no-stimulation</th>
<th>P_{close} stimulation</th>
<th>Stimulation vs. no stimulation t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 1 (cm H_{2}O ± S.E.)</td>
<td>−42.13 ± 13.63</td>
<td>−42.72 ± 14.86</td>
<td>p &lt; 0.44</td>
</tr>
<tr>
<td>R^2</td>
<td>0.65</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Region 2 (cm H_{2}O ± S.E.)</td>
<td>−15.30 ± 1.29</td>
<td>−20.40 ± 2.20</td>
<td>p &lt; 0.02</td>
</tr>
<tr>
<td>R^2</td>
<td>0.93</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Region 3 (cm H_{2}O ± S.E.)</td>
<td>−8.58 ± 1.19</td>
<td>−9.46 ± 0.54</td>
<td>p &lt; 0.17</td>
</tr>
<tr>
<td>R^2</td>
<td>0.88</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Region 4 (cm H_{2}O ± S.E.)</td>
<td>−6.05 ± 0.91</td>
<td>−7.34 ± 0.77</td>
<td>p &lt; 0.03</td>
</tr>
<tr>
<td>R^2</td>
<td>0.86</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Region 5 (cm H_{2}O ± S.E.)</td>
<td>−4.78 ± 0.50</td>
<td>−6.69 ± 1.44</td>
<td>p &lt; 0.19</td>
</tr>
<tr>
<td>R^2</td>
<td>0.89</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Region 6 (cm H_{2}O ± S.E.)</td>
<td>−4.21 ± 0.76</td>
<td>−3.95 ± 0.61</td>
<td>p &lt; 0.18</td>
</tr>
<tr>
<td>R^2</td>
<td>0.93</td>
<td>0.90</td>
<td></td>
</tr>
</tbody>
</table>

Linear regression for each nasopharyngeal region (region 1 is most rostral, region 6 most caudal) on the pressure vs. CSA relationship was used to obtain mean (n = 4 cats) P_{close} (pressure in cm H_{2}O at CSA = 0) and R^2 value (best linear fit is 1.0) for no stimulation (no genioglossus stimulation) and stimulation (with genioglossus stimulated conditions). Comparison of no stimulation P_{close} to stimulation P_{close} by t-test (fourth column) shows the unadjusted p-values. Bonferroni adjusted significance would be at p < 0.007.

4. Discussion

Extending previous results from this laboratory on pharyngeal airway compliance under passive, unstimulated conditions (Brennick et al., 1998), the current study determined the mechanical effects of selective activation of the genioglossus muscle on pharyngeal CSA, shape and compliance in anesthetized, paralyzed cats. This is the first study to use stimulus-gated MRI to examine the regional effects of intramuscular genioglossus muscle stimulation on pharyngeal pressure-area relationships in anesthetized, paralyzed cats. Genioglossus stimulation significantly increased nasopharyngeal CSA when all CSA results across pressure levels and pharyngeal regions were analyzed, and, within specific regions, there was a trend for a significant increase in CSA in the mid-pharynx (regions 3 and 4) with stimulation. The increase in CSA with stimulation shifted the pressure versus CSA curve upwards and to the left so that the estimated P_{close} was lower in most regions, although this change did not reach significance in any individual regions. Despite its dilating effect, genioglossus muscle stimulation did not change pharyngeal airway compliance as evidenced by the similar slopes of the pressure versus CSA relationships with and without stimulation (Fig. 5). Finally, airway shape in the mid pharynx became more circular with either increased airway pressure or genioglossus stimulation.

Previous studies report that genioglossus activation dilates the pharynx whether by direct muscle stimulation (Strohl et al., 1987; Miki et al., 1989; Schwartz et al., 1996; Isono et al., 1999b) or stimulation of the hypoglossus nerve that innervates the genioglossus (Schwartz et al., 1993; Hida et al., 1995;
Fig. 6. Mean ± S.E. elliptical ratio (lateral airway diameter/anteroposterior airway diameter) vs. mean airway pressure at the six nasopharyngeal regions with (▲) and without (●) genioglossus stimulation. Each panel NP1–NP6 represents regions 1–6, respectively. See text for description of significant effects in specific regions.

Fuller et al., 1999; Kuna and Brennick, 2002; Brennick et al., 2004). Comparison of these results is made difficult by the different stimulation techniques. Stimulation of the hypoglossal nerve (medial branch) activates not only the genioglossus muscle but also the geniohyoid (another tongue protruder) and intrinsic tongue muscles (O’Reilly and Fitzgerald, 1990; Schwartz et al., 1993; Eisele et al., 1995; Fuller et al., 1999; Mu and Sanders, 1999). Previous studies that examined the mechanical effect of pharyngeal muscle activation on the pharyngeal airway have also used a variety of measurement techniques. Studies that measured the effects of stimulation on pressure or volume changes in the isolated upper airway (Strohl et al., 1987; Hida et al., 1995) or critical airway pressure (Eisele et al., 1995; Fregosi and Fuller, 1997) were unable to examine the effects at precise regions of the mechanically heterogeneous pharyngeal airway. While fiberoptic imaging is able to evaluate the effect of pharyngeal muscle activation at specific airway regions, technical limitations markedly restrict the number of pharyngeal regions that can be evaluated with this method of imaging (Brennick et al., 1997; Isono et al., 1999b; Kuna and Brennick, 2002; Kuna, 2004). Thus, one of the advantages of the MR imaging technique used in the current study is its ability to localize findings to specific regions of the pharynx (Brennick et al., 1998).

An earlier report from this laboratory provides evidence that our electrical stimulus did not spread to the adjacent geniohyoid muscle (Brennick et al., 1997). In that study (Brennick et al., 1997), intramuscular electrodes were implanted in both the geniohyoid and genioglossus muscles and comparison of sagittal images showed that geniohyoid stimulation caused “buckling” of the tongue, i.e. the tongue’s middle surface rose into the oral cavity, while genioglossus stimulation showed only ventral and anterior movement of the tongue. In the current study tongue “buckling” was not observed, thus providing some assurance that current spread to the geniohyoid did not occur. The MR images in the current study were similar in contrast and quality when compared to our previous MRI study in cats (Brennick et al., 1998). This suggests that there was not a substantial increase in RF noise due to the stimulating electrodes and suggests that using the PSIU6 photo-isolation unit and shielded cabling was effective in preventing RF noise artifact from the stimulating source. There is also good evidence for both the reproducibility of the muscle mechanical action and muscle viability since the images had no noticeable motion artifact from the gated MRI protocol that required 512 stimulation cycles for a typical acquisition.

The use of the paralytic drug gallamine triethiodide that blocks transmission of a neural stimulus at the neuromuscular junction assured that other pharyngeal muscles were not tonically or phasically active during the experiments. Although, we have previously found that in the cat pharynx (Brennick et al., 1998) that there was not a statistical difference in compliance in the non-paralyzed and paralyzed conditions, those results did not measure muscle activity (EMG) in the two preparations so the degree to which muscle activity may have been present in the non-paralyzed preparation was unknown. In another approach, Isono et al. (1999b), used anesthesia with a muscle relaxant during genioglossus muscle stimulation to examine collapsibility in the pharynx. In the current study, the anesthetized paralyzed preparation allowed us to selectively stimulate the genioglossus muscle knowing that activity in other pharyngeal muscles was unquestionably abolished. However, although we did not test stimulation in paralyzed and non-paralyzed conditions, it is possible that there was incomplete stimulation of the genioglossus in the paralyzed preparation or that more complete muscle fiber stimulation may have resulted if a non-paralyzed preparation was used.
In a recent fiberoptic imaging study in spontaneously breathing decerebrate cats, Kuna and Brennick (Kuna and Brennick, 2002; Kuna, 2004) found that the oropharyngeal airway across a pressure range similar to that used in the current study, was patent under unstimulated conditions and that medial branch hypoglossal nerve stimulation enlarged the oropharynx and decreased pharyngeal airway compliance. This result is somewhat contrary to the current study where intramuscular stimulation resulted in nasopharyngeal enlargement, with no compliance changes while the oropharyngeal airway was generally closed under unstimulated conditions at atmospheric and negative pressures. Although in one or two cats stimulation did open or enlarge the oropharynx in the mid-pharyngeal region, because of the overall variability of results in the oropharynx we could not draw conclusions about the effects of genioglossus stimulation on the oropharynx in the current study. Possible reasons to explain the difference between in oropharyngeal results between the current and previous (Kuna and Brennick, 2002) studies are: (1) the intramuscular electrodes in the rostral part of the oral sulcus (Fig. 3) may have affected tongue movement in relation to the oropharynx, (2) surface adhesion forces may have prevented oropharyngeal opening (Kirkness et al., 2003) or (3) cats were paralyzed and mechanically ventilated in the current study but were spontaneously breathing in the previous study (Kuna and Brennick, 2002). However, the difference in that no compliance changes found in the current study and reduced compliance was found in the previous study (Kuna and Brennick, 2002) are probably related to the different stimulation techniques used i.e., intramuscular versus medial hypoglossal nerve stimulation.

Although a range of intensities were used to assess the optimal stimulation levels, our studies did not provide data that could be used to compare nerve and intramuscular responses from a stimulus-dose response curve. We do know, that medial nerve stimulation has been shown to innervate both the genioglossus and geniohyoid and intrinsic tongue muscles, while intramuscular stimulation in the paralyzed cat was probably limited to the genioglossus and intrinsic tongue muscles (O’Reilly and Fitzgerald, 1990; Schwartz et al., 1993; Eisele et al., 1995; Fuller et al., 1999; Mu and Sanders, 1999). This is important since the geniohyoid is thought to brace the hyoid bone and stabilize the pharyngeal airway (Roberts et al., 1984; van Lunteren et al., 1987a). Finally, comparison of results from Kuna and Brennick (Kuna and Brennick, 2002) to the current study suggests that stimulation of genioglossus and geniohyoid through the medial nerve would be more advantageous to airway stability since both $P_{\text{close}}$ and compliance were reduced whereas, intramuscular stimulation of the genioglossus alone caused only airway dilation without a reduction in compliance.

Previous studies have used a variety of techniques to assess pharyngeal airway collapsibility: pressure–area relationships, pressure–volume relationships, closing pressure, and critical airway pressure. Pressure–volume (area) relationships are the standard techniques to measure airway compliance. As pointed out by Horner (Horner, 1996), an intervention can alter $P_{\text{close}}$ (a measures of pharyngeal collapsibility) without a change in airway compliance. That is a decrease in $P_{\text{close}}$ could result from an upward, parallel shift of the pressure versus CSA relationship with no change in compliance, or from a reduction in the slope of the pressure versus CSA relationship, i.e., decreased compliance. Several studies have used pressure changes (Strohl et al., 1987) or volume changes (Hida et al., 1995) to evaluate $P_{\text{close}}$ and compliance. Although Hida and his colleagues (Hida et al., 1995) studied the effects of only positive pressures, they found that hypoglossal nerve stimulation decreased airway compliance. However, in agreement with the findings of the current study, Strohl et al. (1987) concluded that direct genioglossus muscle stimulation lowered the closing pressure by dilating the upper airway rather than by decreasing compliance.

Decreased collapsibility without a change in compliance was noted in the current study by lowering of $P_{\text{close}}$ due to stimulation that trended towards significance in the mid-pharyngeal regions. The modest response reflected in lowered $P_{\text{close}}$ was not entirely unexpected since we used a paralyzed preparation that was designed show only the effects of genioglossus muscle stimulation. In a previous MRI study in cats (Brennick et al., 1998), we found that compliance varied from rostral to caudal nasopharyngeal regions and that $P_{\text{close}}$ varied from approximately $\pm 12.0$ cm H$_2$O rostrally to $\pm 2.5$ cm H$_2$O caudally. These values are comparable to the rostral to caudal (unstimulated) $P_{\text{close}}$ found in the current study (Table 1). More specifically, in the previous study (Brennick et al., 1998) nasal pressures in spontaneously breathing anesthetized cats the ranged from approximately $\pm 6.0$ cm H$_2$O rostrally to $\pm 1.0$ cm H$_2$O suggesting that overall magnitude of the change in $P_{\text{close}}$ $\pm 1.56 \pm 0.72$ cm H$_2$O found in the current study was in the physiologic range of pharyngeal pressures in anesthetized cats and these values are on the same order of magnitude as the decrease in $P_{\text{crit}}$ during genioglossal stimulation (3.2 $\pm 2.5$ cm H$_2$O (Oliven et al., 2003) found in sleeping humans. Previous studies that tested, increased tracheal tension (Thut et al., 1993), tongue protrusion (Rowley et al., 1996) or reflex responses to CO$_2$ (Seelagy et al., 1994) found collapsibility (by decreased $P_{\text{crit}}$ in the isolated cat airways) was reduced by a larger magnitude than the reduction in $P_{\text{close}}$ in the current study. Thus, not withstanding differences in methodology, it could be inferred that in comparison with mechanical and reflex interventions (Thut et al., 1993; Seelagy et al., 1994; Rowley et al., 1996), genioglossus stimulation in the current study was indeed limited to the singular effects of that muscle and that more global mechanical or reflex interventions may have a greater effect on pharyngeal patency.

Our findings are consistent with a clinical study that evaluated the effects of intramuscular and medial hypoglossal medial nerve stimulation on $P_{\text{crit}}$ in OSA patients during NREM sleep (Oliven et al., 2003). They found that with either intramuscular stimulation or single side hypoglossal nerve stimulation there was an overall decrease in $P_{\text{crit}}$ (3–4 cm H$_2$O) associated with increased VImax and improvements but not complete elimination of apnea in those patients (Oliven et al., 2003). However, their analysis of the pressure flow curves suggested that the muscle or nerve stimulation resulted in a ‘purely dilating effect on the airways’ i.e., without a change in compliance. Thus, while the measures used in that study (Oliven et al., 2003) differ from
those in the current study, their conclusion that genioglossus stimulation dilated but did not alter airway compliance is similar to that found in the current study.

Our finding that the ratio of lateral to AP airway diameter increased with pressure or genioglossus stimulation in the mid-nasopharynx (regions 2−5) implies that the AP diameter of the airways increased more than the lateral diameter in those regions. While it is likely that increases in both AP and lateral diameters occurred, the results imply that the AP increased relatively more than lateral dimensions during stimulation and/or increased static pressure application. However, this does not necessarily mean nasopharyngeal airway walls in the AP direction are more compliant since the shape change may be a result of AP pharyngeal wall displacement with elastic stretch or perhaps folding of more flexible lateral walls. In studies in the rabbit pharynx, Kairaitis et al. (2003, 2006) suggests that mechanical changes (neck flexion or mandibular advancement) alter the measured extraluminal tissue pressure surrounding the airway and this can contribute to a reduction in pharyngeal collapsibility. Thus, it could be said that genioglossus stimulation lowered the extraluminal tissue pressure of the tongue on the soft palate resulting in airway dilation and predominantly AP diameter increases. There is also evidence in rats using MRI with tissue tagging that pharyngeal wall tissue displacement during medial hypoglossal nerve stimulation increases AP diameter with minimal or negative (airway center directed) lateral wall displacement (Brennick et al., 2004). In the current study, the shape of the nasopharynx in all regions with the long axis in the lateral directions (Fig. 6, elliptical ratio >1) is consistent with a model for normal human airway behavior and orientation suggested by Leiter (1992, 1996) wherein, the greatest efficiency of genioglossal activation was predicted for elliptically shaped airways. Since cats are not known to exhibit OSA, it seems reasonable that an oblate elliptical orientation (long-axes in lateral direction) was found and that genioglossus stimulation did in fact increase CSA, as predicted by Leiter for normal human airways (without OSA), by increasing preferentially the AP versus the lateral diameter (Leiter, 1996).

Isono et al. (1999b) reported that bilateral transmucosal electrical stimulation of the tongue lowered the compliance of the oropharyngeal segment near the epiglottis in anesthetized patients with OSA. Besides the obvious species-related differences in the anatomy of the cat and human airway, several differences between that study (Isono et al., 1999b) and the current study may account for the different findings with regard to compliance change due to muscle stimulation. First, OSA patients had predominately positive closing pressures (high collapsibility) whereas, cats, having no OSA pathology, maintain patency at negative pressures (Fig. 5, all nasopharyngeal regions patent at −5.0 cm H2O). Secondly, Isono and colleagues’ experiments (Isono et al., 1999b) used high voltage (20−30 V) stimulation in the anesthetized tongue of the patients that the authors acknowledged was likely to have contracted both protruder (genioglossus, geniohyoid) and retractor (styloglossus and hyoglossus) muscles as well as intrinsic muscles of the tongue during the stimulation period. In contrast, in the current study relatively low intensity stimulation was applied directly to the genioglossus through the electrodes at the frenulum of the tongue. Finally, the current study produced static pressure levels in an isolated upper airway that were independent of lung volumes, whereas, Isono et al. (1999b) controlled pressure levels in the upper airway but did not control lung volume changes. Thus, possible airway compliance changes in that study (Isono et al., 1999b) could have resulted from reduced lung volume changes that alter tracheal tension (van Lunteren et al., 1987b; Van de Graff, 1988; Rowley et al., 1996).

In summary, we found that there was a significant overall effect of genioglossus stimulation on CSA in the nasopharynx and that airway dilation occurred over a range of negative and positive static pressure levels in the isolated, sealed upper airway of paralyzed cats. Airway dilation with stimulation, although significant throughout the nasopharynx, was noted as a strong trend in two specific regions of the mid-nasopharynx. We found that the elliptical ratio in the mid-nasopharyngeal regions decreased with increases in airway pressure and with stimulation, yielding a more circular airway. Given that the pharynx is a complex structure, the results of the current study support a role for the genioglossus as a mechanical dilator of the nasopharynx, and provide evidence that selective intramuscular stimulation of the genioglossus alone does not alter pharyngeal airway compliance.

Acknowledgements

The authors gratefully acknowledge Dr. Sam Kuna for advice in reviewing the manuscript. Support by: NIH HL07713, NIH HL42236, NIH EB1780 and NIH HL27520.

References


Kuna, S.T., 2004. Regional effects of selective pharyngeal muscle activation on
Kirkness, J.P., Christenson, H.K., Garlick, S.R., Parikh, R., Kairaitis, K., Wheat-
Isono, S., Tanaka, A., Nishino, T., 1999b. Effects of tongue electrical stimulation
Hida, W., Kurosawa, H., Okabe, S., Kikuchi, Y., Midorikawa, J., Chung, Y.,
Kushima, T., Shirato, K., 1995. Hypoglossal nerve stimulation affects the
pressure–volume behavior of the upper airway. Am. J. Respir. Crit. Care
Horner, R.L., 1996. Motor control of the pharyngeal musculature and implica-
Isomoto, S., Shimada, A., Tanaka, A., Tagaito, Y., Utsumi, M., Konno, A., Nishino,
T., 1999a. Efficacy of endoscopic static pressure/area assessment of the
passive pharynx in predicting uvulopalatopharyngoplasty outcomes. Laryngoscope 109, 769–774.
Isomoto, S., Tanaka, A., Nishino, T., 1999b. Effects of tongue electrical stimulation on
Kairaitis, K., Parikh, R., Stavrinou, R., Garlick, S., Kirkness, J.P., Wheatley, J.R.,
Amis, T.C., 2003. Upper airway extraluminal tissue pressure fluctuations
during breathing in rabbits. J. Appl. Physiol. 95, 1560–1566.
Mandibular advancement decreases pressures in the tissues surrounding the
upper airway in rabbits. J. Appl. Physiol. 100, 349–356.
Kirkness, J.P., Christenson, H.K., Garlick, S.R., Parikh, R., Kairaitis, K., Wheat-
ley, J.R., Amis, T.C., 2003. Decreased surface tension of upper airway
mucosal lining liquid increases upper airway patency in anaesthetised
rabbits. J. Physiol. 547, 603–611.
Kuna, S.T., 2004. Regional effects of selective pharyngeal muscle activation on
Whitelaw, W.A., Isomoto, S., Remmers, J.E., 1993. Site of pharyngeal nar-
Leiter, J.C., 1992. Analysis of pharyngeal resistance and genioglossal EMG
activity using a model of orifice flow. J. Appl. Physiol. 73, 576–583.
Leiter, J.C., 1996. Upper airway shape. Is it important in the pathogenesis of
Littell, R.C., Milliken, G.A., Stroup, W.W., Wolleninger RD, 1996. SAS @ System
McGibney, G., Smith, M.R., 1993. An unbiased signal to noise ratio measure-
Miki, H., Hida, W., Chonan, T., Kikudhi, Y., Takishima, T., 1989. Effects of
submental electrical stimulation during sleep on upper airway pressure in
electrical stimulation of the tongue during wakefulness and sleep. Respir.
Physiol. 127, 217–226.
Olivain, A., O’Hearn, D.J., Boudewyns, A., Oded, M., DeBacker, W., vande-
Heyning, P., Smith, P.L., Eisele, D.W., Allan, L., Schneider, H., Testerman,
genioglossus in obstructive sleep apnea. J. Appl. Physiol. 95, 2023–2029.
function of sternohyoid and sternothyroid muscles in the rabbit. J. Appl. Physiol. 57, 1790–1795.
Rodenstein, D.O., 1992. Assessment of uvulopalatopharyngoplasty for the treat-
ment of sleep apnea. Sleep 15, S56–S62.
tracheal and tongue displacement on upper airway flow dynamics. J. Appl.
Physiol. 80, 2171–2178.
Schwab, R.J., Kuna, S.T., Remmers, J.E., 2005. Anatomy and physiology of
upper airway obstruction. In: Kryger, Roth, Dement (Eds.), Principles and
840–858.
Schwartz, A.R., Thut, D.C., Tuss, B., Seelagy, M., Yuan, N., Brower, R.G.,
the hypoglossal nerve on airflow mechanics in the isolated upper airway.
Schwartz, A.R., Eisele, D.W., Hari, A., Testerman, R., Erickson, D., Smith, P.L.,
Schwartz, A.R., Bennett, M.L., Smith, P.L., De Backer, W., Hedner, J.,
Boudewyns, A., Van de Heyning, P., Ejnell, H., Hochban, W., Knaack, L.,
Podszus, T., Penzel, T., Peter, J.H., Goding, G.S., Erickson, D.J., Testerman,
R., Ottenhoff, F., Eisele, D.W., 2001. Therapeutic electrical stimulation of
of uvulopalatopharyngoplasty in unselected patients with mild obstructive
1993. Tracheal and neck position influence upper airway airflow dynamics
by altering airway length. J. Appl. Physiol. 75, 2084–2090.
Physiol. 65, 2124–2131.
van Luneteren, E., Haxhiu, M.A., Cherniack, N.S., 1987a. Mechanical function
van Luneteren, E., Haxhiu, M.A., Cherniack, N.S., 1987b. Relation between upper
airway volume and hyoid muscle length. J. Appl. Physiol. 63, 1443–1449.
Weaver, T.E., 2002. Adherence to continuous positive airway pressure treatment
Sleep Apnea: Pathogenesis, Diagnosis, and Treatment. Marcel Dekker, Inc.,
New York, pp. 523–554.
582–590.