Mechanisms contributing to the response of upper-airway muscles to changes in airway pressure

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Carberry JC, Hensen H, Fisher LP, Saboisky JP, Butler JE, Gandevia SC, Eckert DJ. Mechanisms contributing to the response of upper-airway muscles to changes in airway pressure. J Appl Physiol 118: 1221–1228, 2015. First published March 6, 2015; doi:10.1152/japplphysiol.01103.2014.—This study assessed the effects of inhaled lignocaine to reduce upper airway surface mechanoreceptor activity on 1) basal genioglossus and tensor palatini EMG, 2) genioglossus reflex responses to large pulses (~10 cmH2O) of negative airway pressure, and 3) upper airway collapsibility in 15 awake individuals. Genioglossus and tensor palatini muscle EMG and airway pressures were recorded during quiet nasal breathing and during brief pulses (250 ms) of negative upper-airway pressure. Lignocaine reduced peak inspiratory (5.6 ± 1.5 vs. 3.8 ± 1.1% maximum; mean ± SE, P < 0.01) and tonic (2.8 ± 0.8 vs. 2.1 ± 0.7% maximum; P < 0.05) genioglossus EMG during quiet breathing but had no effect on tensor palatini EMG (5.0 ± 0.8 vs. 5.0 ± 0.5% maximum; P = 0.97). Genioglossus reflex excitation to negative pressure pulses decreased after anesthesia (60.9 ± 20.7 vs. 23.6 ± 5.2 μV; P < 0.05), but not when expressed as a percentage of the immediate prestimulus baseline. Reflex excitation was closely related to the change in baseline EMG following lignocaine (r² = 0.98). A short-latency genioglossus reflex rapid increases from negative to atmospheric pressure was also observed. The upper airway collapsibility index (%difference) between nadir choanal and epiglottic pressure increased after lignocaine (17.8 ± 3.7 vs. 28.8 ± 7.5%; P < 0.05). These findings indicate that surface receptors modulate genioglossus but not tensor palatini activity during quiet breathing. However, removal of input from surface mechanoreceptors has minimal effect on genioglossus reflex responses to large (~10 cmH2O), sudden changes in airway pressure. Changes in pressure rather than negative pressure per se can elicit genioglossus reflex responses. These findings challenge previous views and have important implications for upper airway muscle control.

negative pressure reflex; genioglossus; tensor palatini; mechanoreceptors; motor impairment

PHARYNGEAL DILATOR MUSCLES are important for maintaining upper-airway patency (37). Multiple factors can modulate upper airway muscle activity. These include sleep-wake state, chemoreceptor and central pattern generator input, lung volume, and negative intrapharyngeal pressure (19, 22, 30, 35, 36, 40, 43, 47). How each of these components influences phasic and tonic activity of the upper airway muscles is incompletely understood. Understanding the mechanisms mediating upper airway muscle activity is important as inadequate activity during sleep can contribute to obstructive sleep apnea (OSA) (6, 15, 34). The current study focuses on the underlying mechanisms that mediate upper-airway dilator muscle activity in response to changes in pharyngeal pressure.

Prior studies indicate that the electromyographic (EMG) activity of genioglossus, the largest upper-airway dilator muscle, increases reflexly in response to activation of surface mechanoreceptors by negative airway pressure (19, 44). Reflex activation during quiet breathing contributes to airway patency (1, 11, 44). Larger decrements (~10 cmH2O and beyond) in upper-airway pressure cause more pronounced short-latency reflex increases in genioglossus EMG (19, 43) that are reduced in size by upper airway anesthesia (18, 44). Initial observations suggested that the genioglossus response to negative pressure was solely excitatory (18, 19, 43, 44). More recent studies using more sensitive neurophysiological approaches revealed an initial short-latency excitation (~20 ms) followed by a secondary state-dependent suppression (~40 ms) evoked by brief pulses of negative pressure to the upper airway (13, 14). The tensor palatini, a palatal dilator muscle, also has a similar short-latency excitation component but no suppression (14). The origin of the genioglossus reflex suppression in humans is unknown. However, it likely reflects active inhibition of the motoneurons rather than disinhibition (13). Genioglossus reflex inhibition is also more pronounced during sleep, and it may play a role in increasing upper-airway collapsibility, particularly during rapid eye movement (REM) sleep (13, 39).

Other phasic respiratory pump muscles (e.g., scalenes, parasternal intercostals, and diaphragm) show short-latency reflex inhibition to respiratory loading that results in transient negative upper-airway pressure (9, 10, 12). The latencies of these inhibitory reflex responses (~30–40 ms) are comparable to the latency of the genioglossus reflex inhibition, suggesting similar mechanisms may be involved (9, 11–14). Reflex inhibition of respiratory pump muscles is not affected by anesthesia of the upper airway and is believed to be mediated by receptors in muscles acting on the chest wall activated by loading (9, 33). However, the effect of upper airway anesthesia on genioglossus reflex inhibition to pulses of negative pressure has not been studied.

Thus, in light of the recent advances in understanding the reflex control of genioglossus and the paucity of tensor palatini muscle data, the mechanisms mediating upper airway reflex control in humans are incompletely understood. Therefore, the current study aimed to determine the effects of topical anesthesia with lignocaine to reduce surface mechanoreceptor input on 1) genioglossus and tensor palatini EMG activity during quiet breathing, 2) genioglossus excitation and inhibition to large pulses of negative airway pressure, and 3) upper-airway collapsibility. We hypothesized that lignocaine would reduce the activity of the phasically active genioglossus muscle but not the tonically active tensor palatini muscle during quiet breathing, 2) reduce the amplitude of the short-latency excita-
were recruited to participate in the study. The protocol was approved by the University of New South Wales Human Research Ethics Committee in accordance with the Declaration of Helsinki (2008). Informed written consent was obtained prior to participation.

**MATERIALS AND METHODS**

**Subjects**

Eighteen healthy men and women \((n = 4)\) not taking any medications with no previous history of upper-airway or respiratory disease were recruited to participate in the study. The protocol was approved by the University of New South Wales Human Research Ethics Committee in accordance with the Declaration of Helsinki (2008). Informed written consent was obtained prior to participation.

**Experimental Setup, Equipment, and Measurements**

Airway pressure and upper-airway muscle electromyography. Following informed consent, the participant’s nostrils were decongested with phenylephrine hydrochloride (Nyal, iNova Pharmaceuticals, Sydney, Australia). On assessment during brief sniff, the more patent nostril was sprayed with anesthesia (co-phenylcaine forte, 5%; ENT Technologies, Melbourne, Australia). Two pressure-tipped catheters (Millar, Houston, TX) were inserted into the anesthetized nostril to the level of the choanae \((P_{cho})\) and epiglottis \((P_{epi})\) to enable measurement of upper-airway collapsibility \((17)\). One catheter was advanced 0.5–1.0 cm distally to the nasopharyngeal wall, at the level of the choanae. The second catheter was advanced 1–2 cm below the base of the tongue, to the level of the epiglottis. Muscle EMG was measured with pairs of bipolar intramuscular electrodes. Following surface anesthesia under the tongue for 5 min \((1\%\) lignocaine), two stainless steel Teflon-coated \((diameter was 127 \mu m bare and 203 \mu m coated)\) fine-wire hooked electrodes with \(-1.5 \text{ mm of the tip removed (no. 791500, A-M Systems)}\) were inserted via a 25-gauge needle into the genioglossus \(-4 \text{ mm either side of the frenulum to a depth of 1-1.5 cm (13)}\). The pterygoid hamulus was located by touch at the junction of the soft and hard palate. Two fine-wire electrodes \((via a 23 \text{ gauge needle})\) were inserted 1–1.5 cm into the palate at a 45° angle along the lateral surface of the pterygoid plate to measure tensor palatini EMG activity \((14)\). To confirm electrode placement and determine the maximum activity of the genioglossus and tensor palatini muscles, the subject was asked to perform tongue protrusions \((pushing the tongue as hard and fast as possible against the top two incisor teeth)\) and several large swallows.

Data were acquired using a 16-bit analog-to-digital converter \((CED 1401; \text{Cambridge Electronic Design, Cambridge, UK})\) and Spike2 software \((version 6.17; \text{Cambridge Electronic Design})\). CED 1902 amplifiers were used to record EMG data at a sample rate of 2,000 Hz with a band pass filter of 30–1,000 Hz. Pressure channels were sampled at 1,000 Hz and the remaining channels at 250 Hz.

Respiratory measures. Subjects were fitted with a nonvented nasal mask \((\text{ResMed, Sydney, Australia})\) connected to a two-way breathing valve \((\text{Hans Rudolph, Series 1410, Kansas City, MO})\). airflow was measured via a heated pneumotachograph \((\text{Hans Rudolph, 3700)}\) and differential pressure transducer \((\text{DP15-16, Validyne Engineering, Northridge, CA})\) and integrated to give tidal volume. An additional differential pressure transducer was attached to the nasal mask to record mask pressure \((P_{mask})\).

Protocol. The study interventions were performed during wakefulness in the supine position. Each subject was studied with and without upper airway anesthesia by inhalation of lignocaine. Under each condition, 5 min of stable quiet breathing was obtained followed by 20 min of quiet breathing in which brief upper-airway negative-pressure pulses of 250-ms duration were delivered to the nasal mask to quantify the genioglossus reflex response and upper-airway collapsibility \((13, 14, 17, 24)\). Pulses were applied every 2–8 breaths during early inspiration via a computer-controlled solenoid \((\text{Asco Numatics, Sydney, Australia}). The solenoids allowed rapid switching of the breathing circuit between room air and an evacuated negative pressure reservoir \((\text{approximately } -100 \text{ cmH}_2\text{O})\). A spring-loaded valve \((\text{HS730-010, Respironics, Sydney, Australia})\) in series limited mask pressure to approximately \(-12 \text{ cmH}_2\text{O}. Nasopharyngeal anesthesia was achieved via inhalation of lignocaine 4% \((\text{PARJ-TIA nebulizer, particle size } > 5 \mu m, \text{PAR})\). Each subject was instructed to breathe though his or her nose for 1 min and switch to oral breathing for another minute, alternating until the lignocaine was fully inhaled \((-10 \text{ minute})\). Prior to baseline and lignocaine conditions, the gag reflex was assessed with the touch of a tongue depressor to the back of the pharyngeal wall. Testing commenced immediately after the gag reflex was abolished during the lignocaine inhalation. At least 1 h after the wakefulness recordings, an overnight sleep study was performed in the sleep laboratory to quantify the apnea/hypopnea index \((\text{AHI})\). Alternatively, participants who elected to not remain in the laboratory overnight performed a home sleep study \((\text{Emblettta 100, Embla or Apnealink, ResMed, Australia})\) on a separate night.

**Data Analysis and Statistical Procedures**

Quiet breathing. Ventilatory parameters including minute ventilation, respiratory frequency, and upper airway resistance were measured on a breath-by-breath basis using customized software. Rectified moving-time averaged \((100 \text{ ms})\) genioglossus EMG was quantified as the peak EMG \((\text{highest value during inspiration})\) and tonic EMG \((\text{lowest value during expiration})\). Tonic EMG was determined for the tensor palatini \((\text{Fig. 1})\). EMG was expressed in absolute units \((\mu V)\) and as a percentage of the maximum activity acquired during maximum maneuvers.

Genioglossus reflex response and upper-airway collapsibility. Customized software \((\text{Spike 2, CED, Cambridge, UK})\) was used to detect the steepest negative slope of \(P_{mask}\) for all the pressure pulses. EMG signals were rectified and averaged for each participant during baseline and lignocaine conditions to quantify reflex responses to negative pressure pulses. Similarly, pressure \((P_{mask}, P_{cho}, \text{ and P}_{epi})\) signals were averaged to assess upper-airway collapsibility and negative-pressure pulse stimulus characteristics. Pulses delivered during a swallow, or where movement or signal artefact occurred, were excluded from analysis. All other pulses in which \(P_{mask}\) was \(< -5 \text{ cmH}_2\text{O}\) were included in the analysis. Stimulus onset \((\text{time zero})\) was determined as the last point before the sudden decrease in \(P_{cho}\). EMG reflex amplitude was expressed as a percentage of the mean prestimulus \((100 \text{ ms})\) EMG and in absolute units \((\mu V)\). To be classified as reflex excitation or inhibition, the rectified averaged EMG had to increase/decrease by at least two SDs \((\pm \text{ the mean prestimulus EMG for greater than 5 ms})\). When present, excitation onset, excitation peak amplitude, inhibition onset, and inhibition nadir were quantified as described previously \((13)\). The area under the curve for the reflex excitation was also quantified and expressed in absolute and normalized terms \((31)\) \((\text{Fig. 2})\). Upper-airway collapsibility was calculated as the pressure difference between nadir choanal and epiglottic pressures during negative pressure pulses as described previously \((17)\). Specifically, the collapsibility index was calculated as: \([P_{cho} \text{ nadir} - \text{P}_{epi}\text{ (at the point of Pcho nadir)}]/\text{Pcho}) \times 100\).

Statistical procedures. Student’s paired t-tests were performed to compare ventilatory parameters, peak and tonic EMG, and the reflex parameters during baseline and lignocaine conditions \((\text{Prism 6, GraphPad Software})\). Data are reported as means \(\pm \text{ SE}\). Linear regression analysis was used to assess the relationship between baseline EMG and reflex peak excitation following negative-pressure pulse application. Statistical significance was defined as \(P < 0.05\).

**RESULTS**

Of the 18 subjects enrolled, data were obtained from 15. One participant could not tolerate the fine-wire electrodes and withdrew from the study, one could not breathe comfortably...
through the nasal mask and could not complete the protocol, and one subject had sleep apnea and struggled to keep their airway open while supine during the lignocaine protocol. Their data were excluded from analysis. For tensor palatini during quiet breathing, data from 14 participants were used as electrodes were displaced prior to lignocaine application in one individual. During large negative-pressure pulses, the fine-wire electrodes from tensor palatini were frequently dislodged. Thus the reflex response to negative pressure in tensor palatini has not been analyzed. For the 15 subjects who completed the protocol, the mean age and body mass index (3 female) was 28 ± 1 yr and 24 ± 1 kg/m², respectively. The mean apnea/hypopnea index was 5.6 ± 2.1 events/hour of sleep.

Effects of Inhaled Lignocaine During Quiet Breathing

Ventilatory parameters and genioglossus and tensor palatini EMG activity during quiet breathing are shown in Table 1. Upper-airway anesthesia had no effect on minute ventilation, inspiratory time, breathing frequency, peak inspiratory flow, or pharyngeal resistance (P ≥ 0.6 for all parameters). However, peak and tonic genioglossus EMG during quiet breathing decreased following lignocaine by ~30%. In contrast, lignocaine had no effect on tensor palatini EMG (Table 1).

Upper-Airway Dilator Muscle Reflex Responses and Collapsibility to Negative-Pressure Pulse Stimuli

Genioglossus EMG reflex responses to negative pressure pulses were obtained in 9 of the 15 participants. The reflex consisted of a short-latency excitation followed by a secondary reflex inhibition present in 4 of the 9 participants. The size, area, and latency of the initial excitation during baseline conditions and after upper-airway anesthesia are shown in Table 2. Mean prestimulus baseline EMG decreased after lignocaine (P = 0.06, Fig. 3A). The absolute amplitude of the
peak excitation decreased following lignocaine inhalation when expressed in microvolts \((P = 0.047, \text{Fig. 3B})\) but not when expressed as a percentage of the immediate prestimulus EMG (Table 2). Similarly, the absolute size of the excitation area decreased after lignocaine but not when normalized to the lower level prestimulus EMG. Changes in the amplitude of the initial genioglossus excitation peak strongly correlated with the changes in prestimulus baseline EMG following lignocaine \((r^2 = 0.98, \text{Fig. 4})\). Airway anesthesia did not alter genioglossus reflex onset latency or the timing of peak reflex excitation. When present \((n = 4 \text{ at baseline, } n = 3 \text{ post-lignocaine})\), lignocaine had a similar effect on the secondary genioglossus reflex inhibition to the initial excitation response. There was a nonsignificant decrease \((n = 3)\) in reflex inhibition amplitude in absolute units following lignocaine \((33.9 \pm 19.7 \text{ vs. } 16.9 \pm 7.9 \mu \text{V}, P = 0.3)\) but not as a percentage of the pre-baseline level \((51.2 \pm 2.6\% \text{ vs. } 53.6 \pm 5.4\%, P = 0.5; \text{see Fig. 2 for example})\). Reflex inhibition onset latency did not differ between baseline and lignocaine \((49.1 \pm 2.7 \text{ vs. } 55.6 \pm 3.2 \text{ ms, } P = 0.3)\).

In 5 individuals of 15, there was a prominent short-latency excitation followed by inhibition in genioglossus upon the return of the negative pressure to atmospheric pressure. The morphology of this reflex response was similar to that observed to the initial negative-pressure stimulus and did not significantly change with lignocaine (Fig. 2, Table 3). Finally, the upper airway collapsibility index to negative-pressure pulses increased following lignocaine anesthesia \((17.8 \pm 3.7\% \text{ vs. } 28.8 \pm 7.5\%, P < 0.05)\).

**DISCUSSION**

The main findings of this study are 1) the input from surface mechanoreceptors of the upper airway increases genioglossus activity, but has minimal effect on tensor palatini during quiet breathing, 2) contrary to our hypothesis, lignocaine does not affect the initial normalized genioglossus reflex excitation to large upper-airway negative pressures, indicating minimal contribution from superficial mechanoreceptors in mediating this response, 3) lignocaine increases upper-airway collapsibility, and 4) rapid changes in airway pressure from negative to atmospheric can produce a short-latency reflex excitation of genioglossus followed by inhibition, similar to the negative pressure reflex.

**Quiet Breathing**

Our observation of decreased genioglossus EMG following inhaled lignocaine is similar to a previous report in which...

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**Table 1. Ventilatory parameters and upper-airway dilator muscle EMG during quiet breathing at baseline and after inhaled lignocaine**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Lignocaine</th>
<th>Change</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minute inspiratory ventilation, l/min</td>
<td>10.7 ± 0.6</td>
<td>10.7 ± 0.8</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Inspiratory time, s</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Breath frequency, breaths/min</td>
<td>14.1 ± 0.9</td>
<td>13.9 ± 0.8</td>
<td>↓ 1%</td>
<td>0.89</td>
</tr>
<tr>
<td>Peak inspiratory flow, l/s</td>
<td>0.6 ± 0.04</td>
<td>0.6 ± 0.1</td>
<td></td>
<td>0.61</td>
</tr>
<tr>
<td>Upper-airway resistance, cmH2O L−1·s−1</td>
<td>2.1 ± 0.5</td>
<td>1.8 ± 0.3</td>
<td>↓ 14%</td>
<td>0.56</td>
</tr>
<tr>
<td>Genioglossus EMG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonic, (\mu \text{V})</td>
<td>16.1 ± 5.4</td>
<td>11.5 ± 4.1</td>
<td>↓ 29%</td>
<td>0.06</td>
</tr>
<tr>
<td>Tonic EMG, % max</td>
<td>2.8 ± 0.8</td>
<td>2.1 ± 0.7*</td>
<td>↓ 25%*</td>
<td>0.03</td>
</tr>
<tr>
<td>Peak, (\mu \text{V})</td>
<td>33.5 ± 9.7</td>
<td>23.1 ± 7.1*</td>
<td>↓ 31%*</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak EMG, % max</td>
<td>5.6 ± 1.5</td>
<td>3.8 ± 1.1*</td>
<td>↓ 32%*</td>
<td>0.01</td>
</tr>
<tr>
<td>Tensor palatini EMG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonic, (\mu \text{V})</td>
<td>7.0 ± 1.0</td>
<td>7.3 ± 1.0</td>
<td>↑ 4%</td>
<td>0.08</td>
</tr>
<tr>
<td>Tonic EMG, % max</td>
<td>5.0 ± 0.8</td>
<td>5.0 ± 0.5</td>
<td></td>
<td>0.97</td>
</tr>
</tbody>
</table>

Values are means ± SE. Upper-airway anesthesia had no effect on ventilatory parameters. Genioglossus \((n = 15; 3 \text{ females})\) peak and tonic EMG decreased after lignocaine, whereas tensor palatini EMG \((n = 14; 3 \text{ females})\) was unchanged. *Significant difference compared with baseline \((P < 0.05)\).
reductions in peak (22%) and tonic (30%) EMG followed upper-airway anesthesia during quiet breathing (44). Indeed, there is further support for the importance of the input from surface mechanoreceptors in contributing to genioglossus activity from experiments in which central pattern generator input is minimized during entrained iron lung ventilation. Under these conditions, phasic genioglossus EMG remains and breath-by-breath peak EMG strongly correlates with negative upper-airway pressure (1, 11). In addition, genioglossus EMG also decreases and upper-airway collapsibility increases when tracheotomized OSA patients breathe via their tracheal stoma compared with via their nose (23).

Based on previous animal and human studies, superficial receptors in the nose, pharynx, or nasopharynx respond to changes in upper airway pressure, flow, temperature, and chemical stimuli, which contribute to many different upper-airway reflexes (4, 5, 21, 45, 46). Nasal pressure receptors respond to negative pressure during airway occlusion via trigeminal afferents (42). Distension of the nasopharynx mucosa activates the receptors in the epithelium that are normally quiescent (32). Receptors in the larynx are silent or discharge tonically and the rate increases or decreases in response to positive and negative pressure applied to the airway (8, 20). Slowly adapting mechanoreceptors in the nasopharyngeal region likely mediate genioglossus EMG increases during sustained negative pressure and EMG decreases during sustained positive pressure applied to the upper airway (28). Vagal feedback from pulmonary stretch receptors inhibits upper-airway muscle activity (3, 21). In our study, although it is possible that mechanoreceptor output from all regions was not completely abolished by lignocaine inhalation, given that both tonic and phasic genioglossus EMG activity decreased with inhaled lignocaine, we conclude that superficial receptors from the nose, pharynx, and larynx clearly contribute to genioglossus muscle activity during quiet breathing.

The current study is the first to examine the effects of lignocaine on tensor palatini EMG during quiet breathing. While increased levels of respiratory stimulation can elicit reflex excitation of tensor palatini (14, 25), the current findings showing no change with lignocaine are consistent with prior observations that pharyngeal pressure at the levels obtained during quiet breathing provide minimal reflex excitation to tensor palatini in most individuals (25). Thus tensor palatini is capable of increasing its activity to increased respiratory loading (14), but the threshold for activation by mucosal mechanoreceptors is higher compared with genioglossus. This observation is interesting given tensor palatini has tonic multifunction activity during quiet breathing, while single unit studies have revealed both inspiratory and expiratory input (34) similar to the genioglossus.

During sleep onset, increased upper-airway resistance is closely related to reductions in tensor palatini EMG (41). Thus the lack of increased upper-airway resistance following lignocaine in the current study, despite reduced genioglossus EMG,

Table 2. Genioglossus reflex excitation at baseline and after lignocaine inhalation

<table>
<thead>
<tr>
<th>Stimulus characteristics</th>
<th>Baseline</th>
<th>Lignocaine</th>
<th>Change</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of replicate pulses</td>
<td>49</td>
<td>40</td>
<td>0.2</td>
<td>0.83</td>
</tr>
<tr>
<td>Pmask, cmH2O</td>
<td>−11.9 ± 0.6</td>
<td>−11.9 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflex excitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset latency, ms</td>
<td>23.1 ± 3.0</td>
<td>22.7 ± 4.7</td>
<td>↓ 1.7%</td>
<td>0.86</td>
</tr>
<tr>
<td>Peak latency, ms</td>
<td>35.5 ± 4.6</td>
<td>36.3 ± 5.9</td>
<td>↑ 2%</td>
<td>0.8</td>
</tr>
<tr>
<td>Peak amplitude, % baseline EMG</td>
<td>190.4 ± 15.9</td>
<td>165.8 ± 16.2</td>
<td>↓ 13%</td>
<td>0.2</td>
</tr>
<tr>
<td>Excitation area, μV·ms</td>
<td>1.11 ± 0.18</td>
<td>0.49 ± 0.08*</td>
<td>↓ 56%*</td>
<td>0.04</td>
</tr>
<tr>
<td>% Area</td>
<td>35.2 ± 6.4</td>
<td>27.9 ± 11.3</td>
<td>↓ 20%</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Genioglossus peak excitation (n = 9) amplitude was reduced in μV after lignocaine. However, it was not different when expressed as % ± prestimulus baseline EMG. Pmask, mask pressure. No. of replicate pulses is the number of pulses averaged for analysis. *Significant difference compared with baseline.

Fig. 3. Genioglossus prestimulus EMG and reflex excitation amplitude scatterplots during baseline and lignocaine. Genioglossus prestimulus EMG (A) and reflex excitation amplitude (B) decreased in μV after lignocaine. Adjacent open symbols indicate means ± SE for each condition.

Fig. 4. Change in genioglossus reflex amplitude strongly correlates with the change in prestimulus baseline EMG following lignocaine. Subjects who had the largest decreases in prestimulus EMG had the largest decrease in reflex amplitude.
may be due to the lack of change in tensor palatini EMG and other upper-airway muscles. Upper-airway anatomy was unlikely to be impaired in the healthy individuals that we studied such that they were not crucially reliant on genioglossus tone for airway patency. A lack of increase in airway resistance with lignocaine in healthy individuals is consistent with an earlier study that also showed reductions in genioglossus activity with lignocaine but no change in upper-airway resistance during wakefulness (44). This may not be the case in people who have lignocaine but no change in upper-airway resistance. This may be due to the lack of change in airway dilator muscles to maintain airway patency. Indeed, lignocaine reduces genioglossus activity in sleep apnea (including electrical stimulation and the need to re-examine the mechanisms mediating positive-pressure breathing to restore airway patency. A lack of increase in airway resistance with lignocaine in healthy individuals may be due to the lack of change in tensor palatini EMG and other upper-airway muscles. Upper-airway anatomy was unlikely to be impaired in the healthy individuals that we studied such that they were not crucially reliant on genioglossus tone for airway patency. A lack of increase in airway resistance with lignocaine in healthy individuals is consistent with an earlier study that also showed reductions in genioglossus activity with lignocaine but no change in upper-airway resistance during wakefulness (44). This may not be the case in people who have lignocaine but no change in upper-airway resistance. This may be due to the lack of change in airway dilator muscles to maintain airway patency. Indeed, lignocaine reduces genioglossus activity in sleep apnea (including electrical stimulation and the development of pharmacological agents to increase genioglossus muscle tone during sleep). Since the short-latency genioglossus excitatory reflex response to negative pressure pulses is unaffected by removal of input from mucosal mechanoreceptors which are known to be activated by negative pressure, then deep receptors (e.g., intramuscular receptors) which are not silenced by upper airway topical anesthesia must be involved. Previous studies have identified muscle spindles in the genioglossus and transverse lingual muscles in humans (27, 38). Alternatively, remote receptors in the inspiratory pump muscles that are sensitive to the change in pressure may reflexly alter the output to genioglossus. Stimulation of intercostal muscle tendon organs reduces the activity of medullary inspiratory neurons (7). Proprioceptive receptors contribute to the short-latency reflex responses to loading in the inspiratory pump muscles (9, 10). Similarly, the genioglossus secondary inhibition was also unaffected by lignocaine. Thus it is possible that genioglossus reflex inhibition is generated by similar mechanisms as the inhibitory reflexes observed in respiratory pump muscles to sudden respiratory loading as the time course is similar, although we cannot conclude this definitively here. Unexpectedly, we observed genioglossus reflex excitation and inhibition when the negative pressure returned to atmospheric pressure. Unloading reflexes (short-latency disfacilitation) have been observed in inspiratory pump muscles at the end of airway occlusion (10, 33). They are thought to result from withdrawal of muscle facilitation of the ongoing contraction following unloading as in limb muscles (2). As inhaled lignocaine also did not attenuate the genioglossus “unloading” reflex, it is possible that similar mechanisms are involved in mediating this response.

Increased collapsibility of the upper airway to negative pressure stimuli after lignocaine despite no change in the size of the normalized genioglossus reflex confirms results from a prior study in which upper-airway collapsibility increased following inhaled lignocaine anesthesia (44). This suggests that the reduction in overall excitability from loss of local mechanoreceptor input still has a role in maintaining airway patency to large deflections in airway pressure.

Methodological Considerations

We were unable obtain reliable tensor palatini recordings in our study during the negative-pressure pulse protocol. Large movement artifacts and frequent dislodgement of electrodes occurred. We hypothesized that lignocaine would reduce tensor palatini reflex excitation to negative pressure pulses. While we were not able to assess this, we were able to make the novel finding that surface mechanoreceptors do not modulate tensor palatini EMG during quiet breathing. It remains uncertain as to whether the reflex excitation in tensor palatini in response to negative pressure pulses are similarly unaffected by minimizing surface mechanoreceptor input. Potential relationships between genioglossus EMG changes with lignocaine and sex, age, BMI, and AHI were not assessed in the current study due to the relatively homogeneous study population. These factors remain important considerations for appropriately designed future investigations. Finally, the current study was designed to investigate the mechanisms mediating upper-airway reflexes in healthy, normal-weight individuals during wakefulness. Thus the current findings may not extend to sleep or to OSA patients.

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Table 3. Genioglossus EMG reflex components at baseline and after lignocaine inhalation on the switch from negative to atmospheric pressure

<table>
<thead>
<tr>
<th>Reflex excitation</th>
<th>Baseline</th>
<th>Lignocaine</th>
<th>Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset latency, ms</td>
<td>14.6 ± 3.8</td>
<td>20.8 ± 3.0</td>
<td>↑ 42%</td>
<td>0.2</td>
</tr>
<tr>
<td>Peak latency, ms</td>
<td>26.7 ± 5.4</td>
<td>25.4 ± 3.1</td>
<td>↓ 5%</td>
<td>0.8</td>
</tr>
<tr>
<td>Peak amplitude, µV</td>
<td>42.8 ± 11.9</td>
<td>29.9 ± 6.1</td>
<td>↓ 30%</td>
<td>0.2</td>
</tr>
<tr>
<td>Peak amplitude, % baseline EMG</td>
<td>156.3 ± 7.7</td>
<td>165.0 ± 6.5</td>
<td>↑ 5%</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5). Lignocaine had no significant effect on genioglossus reflex excitation when the pressure was switched from negative pressure back to atmospheric pressure.
Summary and Implications

The findings of this study indicate that the mechanisms mediating upper-airway reflexes to changes in airway pressure are more complex than previously thought. Specifically, feedback from superficial mechanoreceptors provides important drive to genioglossus but not tensor palatini during quiet breathing when pressure changes are small. However, one or more reflex pathways influence genioglossus EMG to large (and more rapid) increases in respiratory loading. Given that upper airway muscle activity contributes to maintain airway patency, these findings have important implications for conditions in which impaired upper-airway dilator muscle activity contributes to upper-airway collapse.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.C.C., H.H., L.P.F., J.P.S, and D.J.E. performed experiments; J.C.C., H.H., and L.P.F. analyzed data; J.C.C., J.E.B., S.C.G., and D.J.E. interpreted results of experiments; J.C.C. prepared figures; J.C.C., H.H., and L.P.F. analyzed data; J.C.C., J.E.B., S.C.G., and D.J.E. approved final version of manuscript; J.E.B. and D.J.E. conceived the study, designed the experiments, and revised the manuscript; J.E.B. and D.J.E. contributed to data collection.

REFERENCES