Geniohyoid muscle function in awake canines

Yokoba, M., H. G. Hawes, and P. A. Easton. Geniohyoid muscle function in awake canines. J Appl Physiol 95: 810–817, 2003. First published April 4, 2003; 10.1152/japplphysiol.00332.2002.—The geniohyoid (Genio) upper airway muscle shows phasic, inspiratory electrical activity in awake humans but no activity and lengthening in anesthetized cats. There is no information about the mechanical action of the Genio, including length and shortening, in any awake, nonanesthetized mammal during respiration (or swallowing). Therefore, we studied four canines, mean body weight 28.8 kg, 1.5 days after Genio implantation with sonomicrometry transducers and bipolar electromyogram (EMG) electrodes. Awake recordings of breathing pattern, muscle length and shortening, and EMG activity were made with the animal in the right lateral decubitus position during quiet resting, CO₂-stimulated breathing, inspiratory-resisted breathing, and airway occlusion. Genio length and activity were also measured during swallowing, when it shortened, showing a 9.31% change from resting length, and the EMG activity increased by 6.44 V. During resting breathing, there was no phasic Genio EMG activity at all, and Genio showed virtually no movement during inspiration. During CO₂-stimulated breathing, Genio showed minimal lengthening of only 0.07% change from resting length, whereas phasic EMG activity was still absent. During inspiratory-resisted breathing and airway occlusion, Genio showed phasic EMG activity but still lengthened. We conclude that the Genio in awake, nonanesthetized canines shows active contraction and EMG activity only during swallowing. During quiet or stimulated breathing, Genio is electrically active but still lengthens during inspiration.

upper airway; swallowing; shortening; muscle contraction; electromyogram

THE GENIOHYOID MUSCLE (Genio) is thought to be an important upper airway muscle during both respiration and swallowing (2, 8, 15). During inspiration, the extrathoracic airway must resist closure when intraluminal pressure decreases. Previous electromyogram (EMG) studies suggest that Genio may regulate the upper airway; swallowing; shortening; muscle contraction; electromyogram

Surgical Implantation

The project was approved by the animal care committee at the University of Calgary. Each mongrel canine had pairs of bipolar fine-wire EMG electrodes and sonomicrometry transducers implanted in the left Genio. This general technique of chronic sonomicrometry and EMG implantation has been described in detail elsewhere (16, 19, 24). Briefly, under general anesthesia, the left Genio was exposed through a neck midline incision and ultrasonic sonomicrometry transducers and EMG wires were implanted between muscle fibers in the center of the midportion of the Genio. On the muscle, immediately adjacent to each pair of transducers, a fine-wire stainless steel bipolar EMG electrode was attached. Afterward, the animals were allowed to recover. All implants were secured by fine synthetic nonfibrogenic sutures (Pro-
lense, Ethicon) and were externalized by a subcutaneous skin tunnel.

Measurement Techniques

All measurements of ventilation and respiratory muscle function were performed with the animals awake and breathing quietly, while lying in the right lateral decubitus position, which placed the implanted Genio in a nondependent position. The animals were studied with the head in a typical, relaxed “neutral” position (snout at 135° to long axis of the spine, with 0° starting from the tail) (42). For reference, if the snout were fully extended rostrally, it would be at 180° to the axis of the spine. The animals were familiar with the location, routine, and personnel of the recordings. The animals breathed spontaneously through a snout mask, which was connected through a one-way valve to a low-resistance open-breathing circuit (<1 cmH2O·L−1·s) that incorporated a pneumotachograph (Fleisch no. 2) and a piezoelectric differential pressure transducer (model 163P01D36, Honeywell Microswitch) connected across the pneumotachograph to provide measurement of inspiratory airflow. Dynamic measurement within the respiratory muscles of the changing distance between the sonomicrometer transducers of each pair was provided by measuring the speed of transmission of ultrasonic waves via a sonomicrometer (model 120, Triton Technology, San Diego, CA) (19). The output signal of the sonomicrometer was offset, amplified, and then sampled to computer.

By using computer software for data acquisition (DataSponge, Bioscience Analysis Software, Calgary, AB, Canada), all signals were monitored in real time on the computer display and simultaneously collected to hard disk on a microcomputer (IBM, Armonk, NY) equipped with a single-board analog-to-digital system (model MIO-16-H-9, National Instruments, Galveston, TX). Inspiratory airflow, length, and EMG of Genio along with ECG were recorded continuously to an IBM personal computer (model 1700, AM Systems, Everett, WA). Power line interference was abolished by careful shielding techniques and the use of differential preamplifiers with a high common mode signal rejection of 110 dB. Thereafter the signal was filtered to attenuate movement artifact and sonomicrometry noise and allow antialiasing filtering, by use of a six-pole, low-pass Bessel filter at 700 Hz (model 746, LT-4, Frequency Devices, Haverhill, MA) and a matching six-pole, high-pass filter at 20 Hz. The EMG signals were further amplified before being sampled by computer at 4 kHz. The EMG signals were moving averaged (model MA-821RSP moving averager, CWE, Ardmore, PA) and integrated by using a leaky integrator with a 50-ms time constant. ECG was obtained concurrently.

Experimental Protocol

Baseline measurements: Swallowing. While the dog remained in the right lateral decubitus position, small aliquots of water were delivered into the mouth with simultaneous recording of Genio length and EMG activity.

Resting and CO2-stimulated breathing. Baseline room air recordings were followed by CO2-stimulated breathing using a modification (18) of the Read rebreathing technique (33). The dog rebreathed a mixture of 6% CO2 and 93% O2 from a 4-liter bag until the observed end-tidal CO2 (ETCO2) was ~9% or until the animal appeared dyspneic to the animal handler.

Breathing against inspiratory resistance or occlusion. While remaining in the right lateral decubitus position, inspiratory-resisted breathing (Resist) was evaluated by recording 1 min of breathing with a moderate external inspiratory flow resistance (~80 cmH2O·L−1·s). Similarly, Genio length and EMG were measured during airway occlusion (Occl) produced by unanticipated complete obstruction of the inspiratory limb of the breathing apparatus, at end expiration.

Analysis of Ventilation, Muscle Length, and EMG

After data acquisition and storage to disk, data were analyzed by using software programs written by one of the authors (P. A. Easton). The flow signal was evaluated for respiratory timing and digitally integrated; minute ventilation, respiratory frequency, tidal volume, inspiratory time, mean inspiratory flow, and inspiratory fraction of respiration were calculated breath by breath.

Using the sonomicrometry data from implanted muscle, the computer algorithm identified Genio length for each breath that corresponded to the onset of inspiratory flow. In each breath, the computer compared this value with the data samples of muscle length in the final third of the preceding expiration and identified the maximum resting end-expiratory muscle length. This baseline resting length of Genio muscles at end expiration in millimeters was termed LR, i.e., resting length, before the onset of inspiration. This length has been termed “length at functional residual capacity” in some previous reports (17, 19). From this resting end-expiratory length, the change in length for each breath was expressed as a percent change from resting length (%LR). In this report, we express increasing length from baseline LR, i.e., lengthening, as positive values of %LR, whereas decreasing length from baseline LR, i.e., shortening, is expressed as negative values ~%LR. Very small changes in muscle length can be identified. Because sonomicrometer resolution is determined by the wavelength of the ultrasound, muscle length change to as little as ±0.04 mm can be detected (29).

Statistical Analysis

Mean values were exported for review to spreadsheet software (Microsoft Excel, Microsoft, Redmond, WA) and to the personal computer version of SAS (SAS version 7.00, SAS Institute, Cary, NC), for statistical analysis (20). Mean values for Genio shortening and EMG during resting breathing and swallowing were compared by using a paired t-test. Mean values for Genio shortening and EMG were tested across the four conditions, including resting breathing, CO2
stimulation, Resist, and Occl by two-way analysis of variance with repeated measures on one factor. Multiple-comparison testing of the mean values for each individual condition was performed by using Duncan’s multiple-range test.

RESULTS

Measurements were made in four chronically instrumented canines with mean weight 28.8 kg (range 27–34 kg). The studies were conducted an average of 1.5 days (range 1–2 days) after Genio chronic implantation of sonomicrometry transducers and EMG electrodes. Signals from Genio transducers and electrodes were of good quality. In this study, we report measurements for Genio length and EMG in four animals during resting breathing, CO2-stimulated breathing, and Resist and in three animals during Occl.

Resting and CO2-Stimulated Breathing

All subjects breathed quietly in the right lateral decubitus position with the head position stable and relaxed. Figure 1A shows a representative trace from one subject. There was no phasic Genio EMG activity or muscle shortening, only flat baseline concurrent with inspiration during room air breathing (Fig. 2).

During CO2-stimulated breathing, Genio length change and EMG activity did not differ significantly from resting breathing (+0.07 ± 0.04% LR and 0.22 ± 0.28 V, respectively, means ± SD) (Figs. 1B and 2). The Genio action during CO2 stimulation is expressed at a mean ET CO2 of 8.43%, as shown in Table 1. At this modest level of CO2 stimulation, for the group, the means ± SD) (Figs. 1C and 4). These values were not statistically different compared with values taken during resting breathing (P < 0.05). These are the first recordings of mechanical action of the Genio during either respiration or swallowing in any awake, non-anesthetized mammal.

Breathing Against Inspiratory Resistance

During Resist, Genio still lengthened minimally (+0.24 ± 0.27% LR) but showed phasic inspiratory EMG activity (1.39 ± 1.24 V) (see Figs. 1C and 4). These values were not statistically different compared with values taken during resting breathing in this small sample. There was no difference in the degree of Genio lengthening and EMG activity between Resist and CO2-stimulated breathing.

During Occl, Genio again lengthened minimally (+0.25 ± 0.19% LR); however, there was a significant increase in Genio EMG activity (3.06 ± 1.60 V) between Occl and either resting breathing or Resist (P < 0.05) (Figs. 1D and 4). There was no difference in the Genio lengthening between Occl and CO2-stimulated breathing. Genio EMG activity during Occl increased significantly more than CO2-stimulated breathing (P < 0.05).

DISCUSSION

Data Summary

The Genio did not exhibit phasic EMG activity during resting or CO2-stimulated breathing, and it lengthened passively during inspiration. Phasic inspiratory Genio EMG activity was seen in Resist and Occl, but the muscle still lengthened passively during inspiration in both conditions. In fact, Genio EMG activity and concurrent muscle shortening was only noted during swallowing in awake canines. These recordings of geniohyoid length in an awake mammal, expressing lack of change or lengthening with respiration and shortening only with swallowing, are unique to this investigation.

Head Position

Head position is an important determinant of upper airway muscle activity (42). These canines were studied in a position that was normal, resting “neutral” of ~135° to the long axis of the spine, typical for a canine lying comfortably on the side. The decubitus position was advantageous in that we were able to keep the head and neck of the canines in a constant yet relaxed position throughout the course of data collection. Head position may also be invoked to explain the differences in activity between this study and previous anesthetized investigations, because presumably the anesthetized animals were supine with their heads extended or hyperextended for intubation.

Swallowing

Swallowing is a complex action involving the tongue, soft palate, larynx, hyoid bones, pharynx, and esophagus (2, 7). Genio is considered one of the suprahyoid muscles responsible for moving the hyoid apparatus during swallowing (28), and indeed previous studies reported Genio EMG activity during swallowing in dogs (15) and in humans with wire electrodes (22, 36). Moreover, in a recent awake study, many suprahyoid muscles, including Genio, myohyoid, and anterior belly of the digastric muscle, were activated selectively during swallowing, with Genio being the most consistently activated muscle (36).

This study is actually the first report of Genio length and shortening during swallowing. We found significant increases in both Genio EMG activity and concurrent muscle contraction during the swallowing maneuver. This is doubly important: it confirms the mechanical function of Genio during swallowing and serves as a positive control for the remainder of our results.

Resting Breathing

Genio activity during respiration seems to be dependent on the experimental preparation and the presence
or absence of anesthesia. Previous studies showed phasic Genio EMG activity during resting breathing in anesthetized canines (39), in anesthetized rabbits (4, 34), and in anesthetized rats (30) with bipolar wire electrodes. For humans, phasic Genio EMG activity during wakeful, resting breathing was reported by using intramuscular wire electrodes as well (45, 46). However, other literature revealed no Genio EMG activity during the inspiratory phase of resting breathing in anesthetized cats (40, 41) and in anesthetized guinea pigs (9, 10). How can we rationalize these different results?

One possible explanation may involve the method of Genio implantation. The Genio consists of two rela-

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**Fig. 1.** Representative traces of geniohyoid length, moving-average electromyogram (EMG), and inspiratory airflow during resting breathing (A), CO₂-stimulated breathing (B), inspiratory-resisted breathing (C), and airway occlusion (D). A: no phasic geniohyoid (Genio) EMG activity or shortening is present during resting breathing. B: no phasic Genio EMG is present during CO₂ stimulation, but Genio lengthens minutely during inspiration. C: phasic Genio EMG is evident during resisted breathing while Genio lengthens during inspiration. D: during inspiratory occlusion, Genio phasic EMG and lengthening.
change per breath; position. The anatomic relationships of Genio provide that Genio and sternohyoid muscles became longer or keep the head position constant during the study. Sive tension on the Genio and making it possible to the right lateral decubitus position, reducing the pas-sive position. In this study, canines were placed in supine position. In this study, canines were placed in.

jects. The anesthetized studies (4, 34, 39, 41) were

mal, the goat, did not show phasic inspiratory activity cle during the awake state from another large mam-

that bipolar EMG recordings from the geniohyoid mus-

contamination from the genioglossus. It is of interest that human Genio is activated differently than other mam-

hypoglossal (XII) nerve in awake cats. Unless the hu-

resting breathing from the geniohyoid branch of the

Lunteren et al. In addition, Umezaki and colleagues

no Genio activity with our

work supports this explanation because we witnessed another source of variability in Genio EMG activity during quiet breathing (K. D. O’Halloran, J. K. Her-

and G. E. Bisgard, personal communication).

Another source of variability in Genio EMG activity during resting breathing is the positioning of the sub-

jects. The anesthetized studies (4, 34, 39, 41) were

performed with the animals’ heads extended, in the supine position. In this study, canines were placed in the right lateral decubitus position, reducing the pas-

sive tension on the Genio and making it possible to keep the head position constant during the study.

Van Lunteren and coworkers (40, 41) also showed that Genio and sternohyoid muscles became longer or did not change length (but never shortened) during resting breathing with anesthetized cats in the supine position. The anatomic relationships of Genio provide insight. Genio extends upward from the hyoid bone to the mandibular symphysis. The sternohyoid muscle arises from the manubrium of the sternum and the first costal cartilage and inserts on the basihyoid (2). During inspiration, the parasternal intercostal mus-

cles move the sternum caudally in canines (11–13), and in conjunction the sternohyoid muscle also moves the hyoid arch caudally. As a result the Genio may be lengthened passively during inspiration (41). Our find-

ings are consistent with this assessment, because we recorded passive Genio lengthening during inspiration.

Implantation and Instrumentation Techniques

When comparing our results in this study with pre-

vious work, any differences should not be attributed to implantation, recovery, or EMG measurement tech-

niques. For example, any differences in the filter band width and EMG Paynter time constant are irrelevant for the other Genio investigations we have cited. A human study that reported phasic Genio EMG activity during wakeful, resting breathing used a band-pass setting of 30–3,000 Hz and a time constant of 200 ms (45, 46), whereas we utilized 20–700 Hz and 50 ms. The filter settings we employed are quite standard in capturing the respiratory muscle EMG spectrum (25, 35). The amount of muscle EMG beyond 700 Hz is minuscule (or nonexistent), and a very high, low-pass setting such as 3 kHz will not capture any appreciable respiratory muscle EMG but does increase the proba-

bility that some extraneous, non-EMG, high-frequency noise will alias down into the recording and artifactu-

ally create “EMG” where none exists. Similarly, a longer time constant of 200 ms will tend to smooth out small EMG signals, making detection more difficult, whereas our 50-ms time constant is likely to preserve even tiny EMG bursts (32). Thus the absence of EMG activity in this study is not an artifact related to EMG measurement technique. Moreover, the implantation techniques and recovery were not confounding. These geniohyoid recordings were conducted 1–2 days after implantation, rather than the 7–10 days we wait before diaphragm measurements. But the 7- to 10-day

Table 1. Breathing pattern

<table>
<thead>
<tr>
<th></th>
<th>Resting Breathing</th>
<th>CO₂-Stimulated Breathing</th>
<th>Resist</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₁, l/min</td>
<td>8.28 ± 5.00</td>
<td>18.10 ± 8.18*‡</td>
<td>3.56 ± 1.19</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>20.70 ± 8.06</td>
<td>23.60 ± 8.95</td>
<td>15.50 ± 5.59‡</td>
</tr>
<tr>
<td>Vᵣ, liters</td>
<td>0.38 ± 0.07</td>
<td>0.75 ± 0.05*‡</td>
<td>0.24 ± 0.07†</td>
</tr>
<tr>
<td>Tᵢ, s</td>
<td>1.26 ± 0.27</td>
<td>1.34 ± 0.34</td>
<td>0.57 ± 0.37</td>
</tr>
<tr>
<td>VT/TI, l/s</td>
<td>0.33 ± 0.15</td>
<td>0.61 ± 0.25*‡</td>
<td>0.12 ± 0.03‡</td>
</tr>
<tr>
<td>Tᵢ/TT</td>
<td>0.41 ± 0.06</td>
<td>0.49 ± 0.04*</td>
<td>0.48 ± 0.05‡</td>
</tr>
<tr>
<td>ETCO₂, %</td>
<td>3.90 ± 0.64</td>
<td>8.43 ± 0.03*</td>
<td>4.06 ± 0.68</td>
</tr>
</tbody>
</table>

Values are mean ± SD for 4 canines. V₁, minute ventilation; f, respiratory frequency; Vᵣ, tidal volume; Tᵢ, inspiratory time; VT/TI, mean inspiratory flow; Tᵢ/TT, inspiratory fraction of respiration; ETCO₂, end-tidal CO₂. *Significant difference between resting breathing and CO₂-stimulated breathing, P < 0.05. †Significant difference between resting breathing and inspiratory-resisted breathing (Resist), P < 0.05. ‡Significant difference between Resist and CO₂-stimulated breathing, P < 0.05.
delay is only related to the lengthy inhibition of the diaphragm that is a feature of laparotomy (1, 16–19, 24). In many years of implantation of many muscles, we have no evidence of any postimplantation inhibition of any muscles other than the diaphragm.

Stimulated Ventilation

Increasing phasic Genio EMG activity during CO2 rebreathing (ETCO2 55 Torr) has been reported in anesthetized dogs in the supine position (39). Similarly, Van Lunteren and coworkers (41) reported Genio EMG activity in anesthetized cats during CO2 rebreathing with a mean CO2 threshold for Genio EMG activity near 49 Torr. However, in this study, awake, nonanesthetized canines did not show phasic Genio EMG activity during moderate CO2-stimulated breathing (ETCO2 56 Torr). It may be of interest that, in both previous studies (39, 41), tracheal tubes traversed the upper airway. We would expect some upper airway muscles to show less EMG activity during anesthesia (4, 6), whereas we recognize that some respiratory muscles, e.g., diaphragm, show relatively greater activity during anesthesia (14) because of anesthetic inhibition of the chest wall and upper airway muscles. Indeed, hypoglossal (XII) nerve activity was depressed much more than phrenic discharge after anesthesia (23). This may indicate that other factors or the combination of factors listed above could account for the differences seen in awake and anesthetized animals.

In anesthetized cats (40, 41), during progressive hypercapnia, there were transient increases in Genio inspiratory lengthening, until a threshold value (56 ± 3 Torr), after which Genio actually started to shorten during inspiration. However, in these awake canines, Genio did not shorten at similar levels of CO2 stimulation (ETCO2 56 Torr). Perhaps there is a species difference in the CO2 threshold value needed for activation and contraction. In addition, Van Lunteren and colleagues (40, 41) demonstrated that the CO2 threshold for Genio EMG was significantly lower (49 ± 2 Torr) than for Genio muscle shortening. Therefore, we should not expect to see any Genio muscle shortening in our study if we had yet not reached the Genio EMG activity threshold.

Inspiratory-resisted Breathing

In anesthetized dogs (39), Genio EMG activity during airway occlusion was increased compared with resting breathing in four of five supine animals. However, in six of eight anesthetized cats (40), Genio EMG activity during occlusion was the same as resting breathing, and there was an elongation of Genio that was attributed to increased caudal movement of the sternum. In addition, the Genio elongation was shown to occur with negative upper airway pressure in anesthetized cats (42), possibly because of decreasing the upper airway pressure reflex (26, 43) because of anesthesia. In this study, Genio also lengthened during Resist in nonanesthetized canines. However, phasic inspiratory Genio EMG activity was detected. So perhaps we did see evidence of an upper airway negative pressure reflex.

![Graph](image1)

Fig. 3. Representative trace of Genio length, EMG, and flow during swallowing. Genio EMG activity with muscle shortening during swallowing. Other conventions as in Fig. 1.

![Graph](image2)

Fig. 4. Group mean geniohyoid length change and EMG during resting breathing, inspiratory resistance, and occlusion. Resist, inspiratory-resisted breathing. Oocl, airway occlusion. ○, Genio length change; ●, Genio EMG. Bars are SE. There was no significant difference in mean Genio lengthening between conditions. *Significant increase in mean Genio EMG activity between Oocl and resting or resisted breathing (P < 0.05).
During Occl we saw significantly more Genio EMG activity compared with Resist; however, under both conditions the muscle lengthened. The Genio is a small, thin muscle (2), incapable of generating sufficient force to overcome the passive tension placed on it, despite increased Genio activation. This may be an example of active eccentric “contraction,” and it underscores the subtlety of the role of Genio in maintaining laryngeal patency. Increased Genio tension during an attempted inspiration in the face of airway occlusion should tend to fix the position of the hyoid bone, and, in conjunction with sternohyoid activity, could help increase upper airway patency (39, 41) even as we measured lengthening. Finally, the Hering-Breuer reflex inhibits upper airway muscle activity (5, 43). The absence of this reflex during Occl might also account for this large increase in Genio EMG activity.

Upper Airway Muscle Coordination

Coordinated activity of both Genio and sternohyoid muscles produces a net force vector to move the hyoid arch in an outward direction, resulting in a much greater dilation of the upper airway than would occur if either muscle was activated alone (39, 41). However, other muscles may play a larger role in upper airway patency. For example, genioglossus muscle stimulation (3, 31) decreases upper airway resistance more than Genio or any other upper airway muscle stimulation, alone or in combination, in supine anesthetized dogs. This should not be surprising given the differences in muscle mass (2, 21, 27) and mechanical orientation of the genioglossus and Genio. Thus the practical effect of the Genio on upper airway patency in nonanesthetized inspiration is probably very small.

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DISCLOSURES

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REFERENCES