Behaviors of hypoglossal hyoid motoneurons in laryngeal and vestibular reflexes and in deglutition and emesis

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Umezaki, Toshiro, Ken Nakazawa, and Alan D. Miller. Behaviors of hypoglossal hyoid motoneurons in laryngeal and vestibular reflexes and in deglutition and emesis. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R950–R955, 1998.—Reflex responses of hypoglossal motoneurons innervating the geniohyoid (GH) and thyrohyoid (TH) muscles from the superior laryngeal (SLN) and vestibular nerves and their behaviors during fictive swallowing and vomiting were examined by recording both the extracellular activities of 11 single cells in the hypoglossal nucleus and GH and TH muscle nerve activity in eight decerebrate, paralyzed, and artificially ventilated cats. The majority of TH motoneurons were either active and/or exhibited shortened antidromic latencies during early expiration. In contrast, GH motoneurons did not exhibit any respiratory-related activity. Electrical single-shock stimulation of the SLN never evoked an excitatory reflex response on GH or TH motoneurons but rather evoked inhibitory responses on the THs. Unlike other hypoglossal motoneurons, GH and TH motoneurons do not appear to receive vestibular inputs. However, they can exhibit robust activities during fictive swallowing and vomiting, particularly during expulsion. Thus these motoneurons may play an important role in airway protection during swallowing and vomiting but not in controlling upper airway patency regulated by vestibular afferents.

thyrhoathyoid muscle; geniohyoid muscle; hypoglossal nucleus; superior laryngeal nerve; vestibular respiratory reflex

THE GENIOHYOID (GH) and thyrohyoid (TH) muscles connect the hyoid bone to, respectively, the mandible and the thyroid cartilage, a main component of the laryngeal framework. These muscles can be involved in various upper airway and digestive behaviors, including swallowing (12, 16), vomiting (9), and vocalization (8). During swallowing in particular, synergistic activation of these hyoid muscles plays a cardinal role both in the closing of the laryngeal vestibule by elevating the entire larynx rostroventrally (12, 16, 22) and in the resulting opening of the upper esophageal sphincter (2, 16). During vomiting, TH motoneurons have been shown to fire phasically in between successive retching bursts and to exhibit vigorously exaggerated activity during the end of the expulsion stage, suggesting a possible mechanism for closing the laryngeal orifice during expulsion similar to that of swallowing (26). The GH muscle is known to exhibit strong activity during expulsion (9).

Apart from such elaborate motor sequences as swallowing and vomiting, more simple and direct “elementary” reflexes from the superior laryngeal nerve (SLN) (3) and vestibular respiratory reflexes in the upper airway musculature (4, 19, 21, 31) may possibly involve GH and TH. These motoneurons are located in the hypoglossal nucleus, except for some TH motoneurons reportedly located in the cat ventral horn at C1 (17). Although there are many reports that hypoglossal motoneurons in general respond to vestibular inputs (13, 14, 19), it is unknown if GH and TH motoneurons in particular are influenced by the vestibular system. In addition, the entire hypoglossal (XII) nerve and some XII motoneurons, which particularly innervate the genioglossal muscle, are known to exhibit inspiratory activity (6, 10, 18). It is very likely that the GH and TH motoneurons also receive respiratory inputs, because the activities of these hyoid muscles greatly affect upper airway patency (28, 30).

Thus, whereas the hyoid muscles can be involved in multifarious behaviors, no attention has been paid to their individual motoneurons in regard to the respective reflex responses and behaviors. In addition to its relevancy for better understanding the functional roles of these muscles in various behaviors, it is relevant for understanding the underlying neural circuitry to know whether the same motoneuron can be involved in different reflexes and behaviors. In this study we examined reflex responses of hypoglossal GH and TH motoneurons from the SLN and vestibular nerve and their behaviors during fictive swallowing and vomiting by recording both extracellular single cell activities in the hypoglossal nucleus and the entire activities of the GH and TH muscle nerves in the decerebrate and paralyzed cat.

METHODS

All the procedures used in this study conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by The Rockefeller University Animal Care and Use Committee.

Data were obtained from eight adult cats of either sex, which were midcollicularly decerebrated under isoflurane anesthesia, paralyzed with gallamine triethiodide, and artificially ventilated to maintain end-tidal CO2 at 4–6%. Neurograms of the TH and GH muscle nerves as well as C5 phrenic nerve, L1 abdominal muscle nerve, and the pharyngeal branch of the vagus (Ph-X), which innervates pharyngeal constrictor muscles, were recorded on the right side using bipolar cuff electrodes to examine the laryngeal and vestibular reflexes and behaviors during fictive swallowing and vomiting. The TH muscle nerve was dissected near the hyoid bone; the GH muscle nerve was dissected beneath the mylohyoid muscle as a branch of the hypoglossal nerve. Both of them were identified using electrical nerve stimulation to elicit a twitch of only the target muscle (26). Unit activities of TH and GH motoneurons were also recorded extracellularly in the hypoglossal nucleus on the right side using glass micropipettes (impedance 3–5 MΩ), and fast green dye was deposited...
electrophoretically through the recording micropipette to localize histologically the recording sites. Recorded cells were identified as TH and GH motoneurons by antidromic activation from the TH and GH nerves, respectively, the same nerves used for recording. The antidromic spikes were confirmed by collision with spontaneous spikes or those elicited during swallowing or vomiting, if possible. Antidromic latency variations (11) were measured at different phases of the respiratory cycle to evaluate possible respiratory inputs to the motoneurons by delivering antidromic stimuli to the TH and GH nerves at 2–5 Hz. During testing of the antidromic latency from the TH and GH nerves, respectively, the same nerve used for recording. The antidromic spikes were confirmed, as described previously (20), to induce vestibular respiratory reflexes, using trains of two to five pulses, 0.2-ms pulse width, 3-ms interpulse interval, repetition rate of one stimulus train every 0.8–0.9 s, delivered at random with respect to the respiratory cycle, at an intensity between 75 and 375 µA (5 times the threshold for evoking a volley recorded from the medial longitudinal fasciculus and always less than the threshold for current spread to the facial nerve, the closest nontarget nerve, as was tested with trains of 50 pulses before paralysis of the animal). The SLN on the right side (ipsilateral to the cell recording side) was stimulated to induce both elementary laryngeal reflexes (3) (using repetitive single pulses of 0.2-ms pulse width at an intensity between 50 and 150 µA, 3–5 times the threshold for evoking inhibitory reflex response on the phrenic nerve, repetition interval 1.3 s delivered at random with respect to the respiratory cycle) and fictive swallowing (using trains of 0.2-ms pulses, 2–30 Hz, 50–200 µA). Fictive swallowing was identified by the burst activity of the Ph-X or TH nerve (5, 26). The vagus nerves were stimulated just rostral to the diaphragm (using trains of 0.3-ms pulses, 25 Hz, 0.5–2 mA) to induce fictive vomiting, which was identified by a characteristic series of bursts of coactivation of phrenic and abdominal muscle nerve discharge (15).

Anesthesia was discontinued after the completion of all surgical procedures and at least 1 h before data collection. The recording data from the nerves and single motoneurons were saved at sampling frequencies of 1 and 50 kHz, respectively, using a Cambridge Electronic Design 1401-plus data interface and Spike 2 software in conjunction with a Power Macintosh 8100/110 computer.

RESULTS

TH and GH nerve activity. During resting respiration, both the GH and TH nerves were usually almost silent. In only three out of eight cats, a weak activity of the TH nerve was observed during the early expiratory phase (Fig. 1A, left). This early expiratory activity of TH was enhanced by stimulating the SLN at an intensity below the threshold for inducing swallowing.

A single shock of electrical stimulus to the SLN elicited overt reflex responses from the phrenic and abdominal nerves as previously described (27), whereas the GH nerve exhibited no response to SLN stimulation at five times the threshold for phrenic nerve inhibition in all six animals tested (Fig. 1A). No response (4 out of 6 cats) or only a weak inhibitory effect (2 out of 6) was observed on the TH nerve (Fig. 1A), but an excitatory response was never seen in response to SLN stimulation.

Vestibular nerve stimulation evoked reflex responses on the phrenic and abdominal nerves that consisted of a combination of increased and decreased neural activity.

![Fig. 1. Typical examples of nerve activities during resting respiration (A, left), fictive vomiting (B, left), and swallowing (B, right) and reflex responses to right superior laryngeal nerve (SLN) (single shock stimuli of 5 times threshold intensity for phrenic nerve inhibition; A, middle) and left vestibular nerve (VN) (trains of 5 pulses at 3-ms interval of 5 times threshold intensity for medial longitudinal fasciculus evoked potential; A, right) stimulation (stim). Large artifacts are seen in synchrony with stimulation pulses. Reflex response traces shown in A, middle, and A, right represent average of 116 and 108 sweeps, respectively. In B, retching phase is delineated by left 2 vertical dashed lines, and beginning and end of expulsion are indicated by solid lines. Stages 1, 2, and 3 of expulsion are also delineated by dashed lines. Fictive swallowing was induced by repetitive SLN stimulation at 2 Hz (B, right). All nerves were recorded on right side. All recordings were obtained in the same cat; gains are indicated in parentheses compared with recording during respiration. Abd, L1 abdominal muscle nerve; GH, geniohyoid muscle nerve; Phr, C5 phrenic nerve; TH, thyrohyoid muscle nerve.]
as previously described (20, 21). In contrast, reflex responses were never evoked on the GH and TH nerves by vestibular nerve stimulation with the same parameters as were delivered to induce vestibular-respiratory reflexes. An example of responses evoked by five shocks of vestibular nerve stimulation is shown in Fig. 1A (right). The absence of short-latency responses that may have been obscured by the five-shock stimulation artifact was confirmed on the oscilloscope and by delivering shorter (2–3 shocks) stimulus trains.

Fictive swallowing, indicated by a burst activity in the TH nerve (Fig. 1B) or Ph-X (Fig. 2, Aa and Ba), was readily induced by electrical stimulation of the SLN at various frequencies from 2 to 30 Hz. The GH nerve also showed a burst activity in synchrony but slightly (20–80 ms) before the TH burst with similar duration (range 350–480 ms) during fictive swallowing, as shown in Fig. 1B.

Vomiting can be divided into a series of retches followed by expulsion. The expulsion phase can be further subdivided into three stages (Fig. 1B). Stage 1 and 2 expulsions are delineated by the presence (stage 1) and then cessation or decline (stage 2) of phrenic nerve activity. The final stage of expulsion (stage 3) is characterized by burstlike exaggerated activity of the TH nerve and Ph-X (Fig. 2, Aa and Ba) (26). The GH nerve exhibited a weak to moderate activity, similar to that of the TH, during the period between successive expulsions (i.e., during the interretch phase). In contrast, the two nerves exhibited different activities during the expulsion phase. The GH nerve exhibited moderate activity beginning at the later part of the stage 1, which was exaggerated during stage 3, whereas TH was activated mainly during stage 3 (Fig. 1B).

TH and GH motoneuronal activity. We recorded unit activities of seven TH and four GH motoneurons, which were antidromically activated from their corresponding nerves with latencies between 1.1 and 1.3 ms (mean ± SD = 1.2 ± 0.1 ms) and 1.0 and 1.7 ms (1.3 ± 0.3 ms), respectively. All the motoneurons were capable of following repetitive stimulation of 300 Hz or more. Antidromic activation was also confirmed by the collision test for all but three cells (2 THs and 1 GH) that showed neither spontaneous discharge nor swallowing or vomiting activities. All TH cells recorded were located in the caudal part of the XII nucleus between 0.6 and 1.2 mm caudal to the obex, whereas GH cells were located more rostrally in the XII nucleus between the level of the obex and 1.9 mm rostral to it. We could not record even antidromic field potentials more caudally in the C1 ventral horn, where some TH motoneurons have been reported (17). None of the TH or GH cells exhibited evoked responses to SLN or vestibular nerve stimulation even though reflex responses were simultaneously recorded on the phrenic and abdominal nerves.

Motoneuronal behaviors observed during fictive swallowing (for all 11 cells) and during fictive vomiting (for 4 GH and 5 TH cells) are summarized in Table 1. Five out of seven TH and three out of four GH cells showed burst activities during fictive swallowing (Fig. 2, Aa and Ba); the remaining three motoneurons were silent. During fictive vomiting, the behaviors of individual motoneurons were more varied than the corresponding whole nerve activities. Three out of five TH cells tested and two out of four GH cells were silent throughout the vomiting period. The behavior of both of the TH motoneurons that were active during vomiting resembled the behavior of the whole TH nerve in that the cells were active both during the interretch period and mainly during stage 3 of expulsion (cf. Fig. 2Aa and Fig. 1B). Whereas the whole GH nerve was mainly active during the interretch phase (Fig. 1B) as well as during expulsion, one GH motoneuron was active during retching bursts (Fig. 2Ba), whereas another was active during the interretch phase. During expulsion, one GH motoneuron fired throughout most of the expulsion period, starting in stage 1, similar to the GH nerve (Fig. 2Ba); the other GH motoneuron was active during stage 2 and 3.

Most of the TH and GH cells were silent during eucapnic resting respiration except for two TH cells...
Table 1. Summary of cell activities

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Symbol –/++ means that cell became active starting during later part of period. Cells 2–4 and 8 were recorded from same animal, as were cells 5 and 11. TH, thyrohyoid; GH, geniohyoid; Spon act, spontaneous activity; Anti, antidromic latency; c-VN, contralateral vestibular nerve; i-SLN, ipsilateral superior laryngeal nerve; R, retching phase; Inter-R, interretch phase; Ex 1, 2, and 3, stages 1, 2, and 3 of expulsion, respectively; ND, not determined; –, no response or silent.

that exhibited weak activities in the early expiratory phase, similar to the activity sometimes observed on the whole TH nerve. However, antidromic latencies were shortened (11) during the early expiratory phase in five out of seven TH cells, including the two with spontaneous expiratory activity, as shown in Fig. 2Aa. In contrast, the latency of the antidromic spike was always invariant in all four GH cells irrespective of the phase of the respiratory cycle during which the antidromic stimulus was delivered (Fig. 2Bb).

**DISCUSSION**

The present study has investigated whether the hypoglossal hyoid, GH and TH, motoneurons are involved in a number of upper-airway behaviors, including swallowing, vomiting, and respiration and SLN and vestibular reflexes. Most of these motoneurons can be multifunctional; however, individual motoneurons differ in regard to the variety of behaviors in which they are involved. The recording sites of the GH and TH motoneurons in our data are consistent with previous anatomic results, with the exception of the possibility of a caudal extension of the TH motor pool into the upper cervical ventral horn. The results of previous anatomic studies were inconsistent concerning the location of TH motoneurons, which were reported to exist in the caudal hypoglossal nucleus and C1 ventral horn (17), the rostral part of the region from the caudal hypoglossal nucleus down into the ventral horn of C2 (7), only in the caudal hypoglossal nucleus of the medulla (25), or only in C1 and C2 (29) or none being observed within the hypoglossal nucleus (24). Although the number of neurons recorded in the present experiment is small, our results suggest that TH motoneurons exist mostly in the caudal hypoglossal nucleus, because even an antidromic field potential from the TH nerve could not be recorded in the C1 ventral horn.

We have recently demonstrated characteristic TH nerve activities during fictive swallowing and vomiting (26). The burst activity of the GH nerve during fictive swallowing is similar to that of TH, although the GH burst starts 20–80 ms before the TH burst. This tendency, which is also observed in actual swallowing (3), can be assumed to be part of the centrally programmed output proper to swallowing because there was an absence of actual movement and proprioceptive sensory feedback in our paralyzed preparation. It was, however, also recognized in our study that swallowing burst activity was not invoked in all GH and TH motoneurons, although a swallowing active motoneuron always exhibited a burst of activity every time swallowing occurred. In contrast to the similarity of the swallowing activities, neural activities during the expulsion phase of fictive vomiting are different between TH and GH motoneurons. GH motoneurons are activated chiefly from the later part of stage 1 expulsion with a transient exaggeration during stage 3, whereas TH motoneurons exhibit a burst activity mainly during stage 3. These findings are consistent with previously described electromyogram (EMG) activities during actual vomiting (9) and suggest the difference of functional roles between the TH and GH muscles during the expulsion phase of vomiting. The GH may assist the opening of the upper esophageal sphincter (2) to facilitate expulsion of vomitus (9) during stage 1 and 2 expulsion. The TH burst activity together with the GH exaggerated activity seems to be important for the closure of the laryngeal vestibule to protect the lower airway during stage 3, when pharyngeal constrictors squeeze the pharyngeal cavity and clean the hypopharynx (9, 26), in a similar manner to that during swallowing (12, 22).

Afferents from the SLN induce airway protective reflexes. A single pulse electrical stimulus to the SLN elicits short latency reflexive EMG activities on upper airway muscles, known as the elementary reflexes (3). Such elementary reflexes were reported to exist not only in laryngeal muscles but also in the GH, TH, and other oropharyngeal muscles in anesthetized animals (3). However, our present results from the decerebrate and paralyzed cat have revealed that a single shock stimulus to the SLN does not evoke excitatory reflex responses corresponding to the elementary reflexes on the GH and TH muscles but rather evokes inhibitory
responses observed only on TH but not GH motoneurons. This finding is consistent with results from a recent EMG study using the decerebrate and spontaneously breathing cat (23).

Vestibular respiratory reflexes are thought to act to maintain airway patency and to compensate for mechanical constraints on respiration that may arise during movements and changes in posture (31). Electrical stimulation of the vestibular nerve produces widespread, bilateral reflex responses that can be recorded from respiratory nerves to upper airway, thoracic, diaphragm, and abdominal muscles (20, 21). The responses typically consist of a combination of increased and decreased respiratory nerve activity. Although there is much evidence that hypoglossal motoneurons receive vestibular inputs (4, 13, 14, 19, 21, 27), the present study strongly suggests that the GH and TH motoneurons are not involved in vestibular-upper airway reflexes. Thus these results indicate that only selected hypoglossal motoneurons innervating certain tongue muscles, e.g., the genioglossal muscle, whose activity can greatly affect upper airway resistance (30), receive vestibular signals.

The present study has also demonstrated that the majority of TH motoneurons receive excitatory inputs during the early expiratory phase, as evidenced by actual motoneuronal discharge and/or the shortening of antidromic latencies (11) during the early expiratory phase. GH motoneurons, on the other hand, did not exhibit any respiratory-related activity. In contrast, other hypoglossal motoneurons are known to have inspiratory activities (6, 10, 18). Although most TH motoneurons were silent during resting respiration in our artificially ventilated preparation, it is possible that more may be recruited to help regulate airway resistance under hypercapnic conditions or other respiratory facilitation (1). Although the functional significance of TH activation during early expiration remains to be clarified, it may help to brake expiratory airflow, similar to the function of the laryngeal adductors during early expiration.

Perspectives

The GH and TH muscles connect the hyoid bone to, respectively, the mandible and the thyroid cartilage, a main component of the laryngeal framework. These muscles may play an important role in airway protection during swallowing and vomiting but not in controlling upper airway patency regulated by vestibular afferents, although TH motoneurons may receive excitatory inputs during the early expiratory phase. Individual TH and GH motoneurons can be multifunctional, but they are not homogeneous in regard to the upper airway behaviors in which they are involved. This study provides distinct evidence that multifunctional cranial motoneurons common to different behaviors other than respiration exist in the mammalian brain stem. However, it remains to be determined whether such motoneurons are driven directly by different central pattern generator (CPG) cells or by common interneurons that belong to different CPGs for individual behaviors. Similarly, we may also need to reevaluate multifunctionality or heterogeneity of other upper airway motoneurons such as cells in the nucleus ambiguus.

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