Motor innervation of the cricopharyngeus muscle by the recurrent laryngeal nerve

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Hammond, Carol Smith, Paul W. Davenport, Alastair Hutchison, and Randall A. Otto. Motor innervation of the cricopharyngeus muscle by the recurrent laryngeal nerve. J. Appl. Physiol. 83(1): 89–94, 1997.—Patients with recurrent laryngeal nerve (RLN) paresis demonstrate impaired function of laryngeal muscles and swallowing. The cricopharyngeus muscle (CPM) is a major component of the upper esophageal sphincter. It was hypothesized that the RLN innervates this muscle. A nerve branch leading from the RLN to the CPM was found in adult sheep by anatomic dissection. Electrical stimulation of the RLN elicited a muscle action potential recorded by electrodes placed in the ipsilateral CPM. Swallowing was investigated by mechanical stimulation of oropharynx pre- and postsectioning of the RLN. Severing of the RLN resulted in a loss of the early phases of swallow-related CPM electromyographic activity; however, late-phase CPM electromyographic activity persisted. The RLN provided motor innervation of the CPM, which also has innervation from the pharyngeal plexus.

swallow; esophageal reflux; glottis

THE LARYNGEAL-PHARYNGEAL COMPLEX is known to be activated during swallowing (3–5, 9, 13, 15). Recurrent laryngeal nerve (RLN) dysfunction has been associated with laryngeal dysfunction, although swallowing dysfunction in patients with RLN paresis (8, 21) has been reported. The cricopharyngeus muscle (CPM) is the most inferior pharyngeal muscle and it inserts on the lamina of the thyroid cartilage and the lateral aspects of the cricoid cartilage. The lower fibers of the CPM initially transverse the dorsal pharynx, connecting to these cartilages, and then distal to the cartilage border the CPM fibers transition to be continuous with the circular fibers of the esophagus (24). The upper esophageal sphincter (UES) is formed by the CPM and its insertions on the thyroid and cricoid cartilages. The CPM and the cartilages comprise the anterior aspect of the sphincter while cervical vertebrae form the posterior aspect (3, 18).

Previously, the CPM has been reported to be innervated by the pharyngeal plexus, formed from branches of the glossopharyngeal, vagus, and sympathetic nerves (24). The RLN is commonly reported to supply the muscles of the larynx, except the cricothyroid and the mucous membrane of the larynx below the vocal folds and the mucous membrane of the upper part of the trachea (24). Lund and Ardran (12) concluded that there was no evidence that the RLN supplied the UES. However, Ekberg et al. (8) and Shin (21), using cineradiographic investigations of patients with suspected paresis of the RLN, demonstrated that impaired laryngeal and pharyngeal functions highly correlated with swallowing dysfunction. Although it is generally accepted that RLN paralysis results in incomplete closure of the larynx, glottic closure during swallowing was not affected by RLN section (22, 23).

Anatomic studies have suggested that the RLN also supplies the CPM. Rustad and Morrison (16) and Steinberg and colleagues (25) found in human cadavers that there were multiple RLN branches of minor and major size and included branches to the pharynx. The main trunk of the RLN was found to divide into two divisions, the cricopharyngeal (CP) nerve and the laryngeal branch of the RLN. The CP nerve was connected to the CPM and the inferior constrictor. However, there are no reports on functional innervation of CPM by the RLN.

The purpose of the present study was to test the hypothesis that the CPM is, in part, anatomically and functionally innervated by the RLN. This was investigated by anatomic dissection of RLN, electrical stimulation of the RLN while recording the electromyographic (EMG) activity of the CPM, and by recording CPM activity during swallowing pre- and post-RLN section. Sheep were used as a model for swallowing in this study because of the anatomic and physiological similarities to humans (1). Anatomic evidence suggests that the sheep and human larynx are similar in the structure of the upper-laryngeal-pharyngeal complex as far as the cartilage and bone structures and the muscles of this area (1). The results demonstrated that the posterior CPM is directly innervated by the RLN.

METHODS

The Animal Care and Use Committee of the J. Hillis Miller Health Center, University of Florida, reviewed and approved the protocol of this study.

Anatomic description of the RLN innervation of the CPM. The anatomic distribution of the RLN in the pharyngeal area was made by postmortem dissection in sheep (n = 12). The neck was dissected to expose the path of the RLN. The branches of the RLN were identified and traced by dissection. The RLN was traced to the CPM. The RLN nerve branch distributed to the CPM was labeled and photographed under a microscope.

Electrophysiological identification of CPM innervation by the RLN in sheep. Functional innervation of the CPM by the RLN was investigated by using electrical stimulation of the RLN while simultaneously recording EMG activity from the CPM in adult sheep (n = 4). The animals were initially anesthetized with pentobarbital sodium (32 mg/kg iv). The sheep was then placed in supine position. The femoral vein
and artery were cannulated for supplemental administration of anesthesia and monitoring of blood pressure, respectively. Lactated Ringer (10% dextrose solution) was continuously provided by slow intravenous infusion. Body temperature was maintained at 38 ± 1°C, with periodic use of a heating pad. The sheep was maintained in a deep plane of anesthesia. Anesthetic state was assessed by blood pressure, eye-blink, and tail-pinch responses.

A midline incision was made in the neck from the thyroid cartilage to the manubrium. A blunt dissection of the omohyoid and sternothyroid muscles exposed the posterior edge of the thyroid cartilage. A suture was placed along the posterior edge of the thyroid cartilage, which was used to rotate the larynx and expose the CPM. Bipolar wire electrodes were sutured into the CPM, between the posterior pharyngeal raphe and CPM insertion on the cricoid cartilage. The electrodes were isolated in the CPM by insulation from surrounding tissue. The RLN was isolated along the tracheoesophageal groove ipsilateral to the CPM containing the recording electrodes. The intact RLN was placed across the bipolar stimulating electrodes that were electrically isolated from the surrounding tissue. The identity of the RLN was confirmed by electrical stimulation (5–500 µA) -elicited contraction of the laryngeal musculature. The CPM EMG activity was amplified, band-pass filtered (300 Hz to 3 KHz), displayed on an oscilloscope, and recorded on magnetic tape. The RLN was stimulated with single pulses of 0.5-ms duration at 1 Hz. The CPM EMG activity and the stimulator trigger pulse were recorded on magnetic tape. A minimum of 128 stimulations were recorded.

Data analysis. The data recorded on magnetic tape were digitized at 3 kHz (Cambridge Electronic Design). The computer stored the digitized EMG activity and stimulus pulse. A minimum of 100 CPM responses were then signal averaged, and latency to muscle-evoked activity was determined. The latency from stimulus presentation to the arrival of the stimulus at the CPM muscle, divided by the length of the nerve from the RLN-stimulating electrode site to the electrodes sutured into the CPM, was used to determine the conduction velocity.

EMG response of the CPM pre- and post-RLN section. Swallowing was elicited in four anesthetized adult sheep by mechanical stimulation of the oropharynx subsequent to the electrical stimulation of the RLN. A blunt instrument was placed in the oral cavity, and the oropharynx was gently probed for three to five strokes until the animal swallowed. Swallowing was identified by characteristic pharyngeal EMG activity that accompanied swallowing and visual observation of the swallowing musculature, which involved the movement of the oral, pharyngeal, and esophageal systems. Only records where swallows were seen to proceed from the laryngopharynx in a peristaltic wave caudally through the esophagus were analyzed. The CPM EMG activity recorded during swallowing was integrated and displayed on a polygraph and magnetic tape for subsequent analysis.

The RLN was then ligated bilaterally at two points and severed between the ligatures. Swallowing was again elicited by mechanical stimulation, and the CPM EMG activity was recorded. The vagus nerve was subsequently sectioned bilaterally at the level of the thyroid cartilage to control for any nerve branches from the vagus nerve that may be involved in the pharyngeal plexus. Swallowing was again evoked, and the EMG response was recorded. The RLN stumps proximal to the CPM were then electrically stimulated, and the CPM activity was recorded.

Data analysis. The mean duration and pattern of the integrated electrical activities recorded from the bipolar recording electrodes sutured into the CPM were compared under both intact and post-RLN section conditions. A one-way analysis of variance was used to analyze differences in duration of four swallows in each of the following conditions: pre-RLN section, post-RLN section, and postvagal section. When required, Scheffe’s R-test and Fisher test of multiple comparisons were utilized to determine which group means differed. Significance level was set at P ≤ 0.05.

**RESULTS**

Anatomic description of the RLN innervation of the CPM. A nerve branch connecting the RLN to the CPM was observed in all sheep (n = 12). A photomicrograph is presented illustrating the gross anatomic identification of the CP branch of the RLN (Fig. 1). This nerve had profuse branches that penetrated the CPM.

Electrophysiological identification of CPM innervation by the RLN in sheep. Electrical stimulation of the RLN elicited a motor action potential in the ipsilateral CPM in all animals tested. Figure 2A illustrates the EMG activity recorded from the CPM with a single stimulus pulse. An average of 115 stimulations elicited from the CPM of the same animal is presented in Fig. 2B. The group mean latency from stimulus pulse to onset of EMG activity was of 3.8 ± 0.5 ms (Fig. 2).

The group mean conduction velocity was 30.5 ± 6 m/s. When the stimulator was turned off, no muscle electrical activity was observed. In one animal, the CPM was dissected and separated from the surrounding musculature with nerve supply intact. The RLN-elicited CPM EMG activity was not different from that observed before CPM separation from surrounding tissue. Several folded layers of surgical plastic (4 mm thick) were then placed between the CPM and the interconnected laryngeal and pharyngeal musculature to provide further isolation of the CPM. EMG activity was again present with RLN stimulation and was not different from previously obtained EMG responses. The CPM EMG activity was also unaltered when the attachment of the CPM was separated by blunt dissection from the thyroid and cricoid cartilages (nerve and blood supply remaining intact).

EMG response of the CPM pre- and post-RLN section. The CPM EMG activity recorded during swallowing in four (n = 4) animals was characterized by an initial large-amplitude burst of CPM EMG activity, followed by lower amplitude, longer duration activity (Fig. 3A). The average duration of the CPM EMG response for a swallow in the intact animal was 0.68 ± 0.06 s. After the RLN was severed, CPM EMG activity during swallowing was characterized by low-amplitude activity only. The initial large-amplitude EMG burst was absent (Fig. 3B).

The average duration of the CPM EMG response during swallowing after RLN section was 0.34 ± 0.03 s. Table 1 compares the swallow duration pre- and post-RLN section. There was a significant difference (P < 0.0001) in the duration of EMG activity before and after RLN section.
CPM electrical activity during swallowing in the intact animal, post-RLN section, and after section of the vagus nerve is presented in Fig. 4. The initial large-amplitude phase of EMG activity was again absent after RLN section (Fig. 4B), and vagotomy produced no additional change in the CPM EMG response (Fig. 4C).

There was a significant difference \( (P < 0.05) \) in the CPM EMG duration between the intact state and both RLN cut and cranial nerve X cut conditions. There was no significant difference found between the RLN cut and vagotomy conditions. Stimulation of the proximal stump of the severed RLN elicited a CPM EMG action potential.

**DISCUSSION**

The results of this study demonstrated that the RLN innervates the CPM anatomically and functionally. Anatomic innervation was demonstrated by identification of a branch of the RLN that entered fibers of the CPM. Functional innervation of the CPM by RLN motor nerve fibers was demonstrated by muscle-evoked activity recorded in the CPM resulting from electrical stimulation of the RLN. Removal of the innervation altered the CPM EMG pattern of a swallow. These findings demonstrate that RLN is one component of the innervation of the CPM and plays an important role in swallowing. During the oropharyngeal swallow, biomechanical events involving intrinsic glottic as well as supra- and infrahyoid muscles take place resulting in the closure of the airway (4, 17–19) and opening of the UES (2, 10). These events include adduction of the true vocal folds and arytenoid cartilages followed by vertical approximation of the adducted arytenoids to the base of the epiglottis. The descent of the epiglottis covers the closed glottis, which closes the laryngeal vestibule. The swallow begins with glottal closure followed by the entire larynx pulled in a ventrorostral direction. This displacement results in the positioning of the closed glottis under the tongue base, away from the path of the food bolus, providing protection against aspiration and facilitating closure of the laryngeal vestibule (20, 26, 27).

During the oropharyngeal phase of swallowing, the UES transiently relaxes and subsequently is pulled upward and forward by the contraction of the suprahyoid muscles that displace the larynx. This traction results in the active opening of the UES, which is also modified by the bolus size (2, 10). Under unimpaired conditions, oropharyngeal swallowing begins with the vocal folds adducted and ends when they return to the resting position (3).

Kahrilas et al. (10) combined videofluoroscopy and manometry to analyze pharyngeal contraction in humans during swallowing. Profound shortening of the pharyngeal muscles during bolus transit through the pharynx eliminated access to the larynx and elevated the UES ventrorostrally. The UES is a major barrier preventing refluxed gastric contents from reaching the upper airway. Cook et al. (2) and Kahrilas et al. (10),
using combined videofluoroscopy and manometry, reported that the physiological high-pressure zone of the UES corresponds in size and location to the CPM. The diameter of the sphincter was directly related to the volumes swallowed. Larger boluses increased the prolongation of the interval of sphincter relaxation. Tonic CPM activity (7) normally present in the awake animal was not observed in the present study, probably due to the use of barbiturate anesthesia and the deep plane of anesthesia. This is supported by the report of a decrease in UES pressure during non-rapid-eye-movement sleep, which was likely due to a decrease in the tonic activity of the muscles of this sphincter (11).

The function of the esophagoglottal closure reflex is to adduct the vocal folds and close the entrance to the trachea in response to abrupt esophageal distention. Shaker et al. (20) reported that the onset of vocal fold adduction preceded the onset of UES relaxation and inferred that the likely efferent fibers controlling the esophagoglottal reflex were located in the RLN and that the target muscles were the glottal adductors. The results of the present study demonstrate the importance of RLN innervation of the CPM for normal swallowing.

Anatomy. The innervation of the CPM by the CP branch of the RLN is similar between humans (25) and sheep (1). Results of the present gross dissection of the laryngeal-pharyngeal area in sheep revealed that a branch of the RLN enters in the muscular wall of the CPM in sheep. Selective stimulation of the RLN elicited EMG activity in the CPM, which can only result from neuromuscular innervation between the motor nerve fibers of the RLN and CPM muscle fibers. The latency and conduction velocities indicate that the motor nerve fibers innervating the CPM are myelinated nerve fibers (9). Sasaki and Isaacson (17) studied fiber types of the RLN at the level where the nerve entered the larynx in the dog and reported that a majority of the RLN axons were myelinated fibers. There were a small number of fibers >16 µm in diameter, and the majority were between the 6–12 µm range. The functional differences in RLN fibers resulted in some fibers allocated for adduction and others for abduction. Diamond et al. (6) reported that the RLN, which supplied the posterior cricoarytenoid muscle in the dog, had three fascicles.
that innervated the three muscle divisions of the posterior cricoarytenoid muscle. These fascicles differed in axon type, composition, and in percentages of sensory, autonomic, and motor fibers. Thus the results of this study support the hypothesis that the RLN innervates the CPM with a branch identified as the CP branch of the RLN.

Physiology. Coordination of the digestive and respiratory systems during swallowing is well established (17, 19). Shaker et al. (20) studied the esophagoglottal reflex, elicited by abrupt esophageal distension, and found the response was characterized by vocal fold adduction, anterior movement of the glottis, opening of the UES and relaxation of the proximal esophagus. The UES and glottis act independently of each other in response to esophageal distension and are likely to be activated by different receptors, but their responses to esophageal distension are complementary. In the present study, the early-phase, large-amplitude CPM EMG response may correspond to fast contraction of the CPM, which supports the musculoskeletal complex of the pharynx in preparation for rapid pharyngeal shortening. In the intact mammal, the CPM may be mediating a shortening of the pharynx with initial large-amplitude activity, followed by sustained closure of the esophagus with low-level tonic CPM EMG activity. Both actions prevent aspiration and/or reflux by anchoring the skeleton to allow for the ventrocranial movement of the larynx to protect the airway and the peristaltic movement of the food bolus down the esophagus by striated musculature. In the case of RLN dysfunction, aspiration can occur because access was allowed to the larynx due to failure of pharyngeal shortening and support of the musculoskeleton in preparation for the ventrocranial lifting of the laryngeal complex and loss of laryngeal adductor motor output. The swallowing CPM EMG response observed in these sheep (Fig. 5) demonstrates the function of the CPM during swallowing and the necessity of RLN innervation; i.e., shortening of fibers that facilitates rapid closure of proximal esophagus and rapid elevation and shortening of the pharynx, allowing the laryngeal area to be protected from infiltration by the bolus (14).

Table 1. Duration of CPM EMG activity during swallows recorded in sheep pre- and post-RLN section

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Swallow EMG duration, s</th>
<th>No. swallows measured</th>
<th>Swallow EMG duration range, s</th>
<th>Mean swallow EMG duration, s</th>
<th>No. swallows measured</th>
<th>Swallow EMG duration range, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.71</td>
<td>4</td>
<td>0.6–0.8</td>
<td>0.38</td>
<td>4</td>
<td>0.3–0.4</td>
</tr>
<tr>
<td>2</td>
<td>0.74</td>
<td>5</td>
<td>0.7–0.8</td>
<td>0.33</td>
<td>5</td>
<td>0.3–0.4</td>
</tr>
<tr>
<td>3</td>
<td>0.63</td>
<td>4</td>
<td>0.6–0.7</td>
<td>0.30</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>0.62</td>
<td>5</td>
<td>0.5–0.7</td>
<td>0.35</td>
<td>5</td>
<td>0.3–0.4</td>
</tr>
</tbody>
</table>

CPM, cricopharyngeus muscle; RLN, recurrent laryngeal nerve.

Fig. 4. Integrated CPM activity recorded during a mechanically stimulated swallow. EMG activity was integrated with a 100-ms time constant. A: integrated EMG activity for 1 swallow in a sheep with RLN and dependent vagus nerve intact. B: integrated EMG activity recorded for 1 swallow after only the RLN was severed in same sheep as in A. C: integrated EMG activity recorded for 1 swallow after RLN and dependent vagus nerve were both severed in same sheep as in A. Arrows indicate onset of the swallow.

Fig. 5. Line drawing demonstrating proposed movement of hypopharyngeal area of the human. Arrows point to direction of movement proposed to occur due to CPM contraction as a result of stimulation by CP branch of RLN. 1: Thyroid cartilage; 2: cricoid cartilage; 3: CPM; 4: CP branch; 5: RLN. [Adapted from Nanson (14).]
Shin et al. (23) suggested that the inferior constrictor muscle (which includes the CPM) functions in cooperation with the intrinsic laryngeal adductor muscles to reinforce glottic closure during swallowing. The repletion of low-level EMG activity after RLN section demonstrates that the CPM has other innervation, presumably from the superior laryngeal nerve. It will be important to further investigate regional differences in the distribution in the posterior CPM for RLN and superior laryngeal nerve nerve fibers. There may be specific differences in the types of motor units that innervate the CPM. It appears from the present results that the RLN fibers mediate the rapid, large-amplitude CPM EMG response and that another pathway mediates the subsequent low-amplitude, sustained CPM EMG response. The RLN may facilitate a rapid closure of the proximal esophagus and rapid elevation and shortening of the pharynx, which allows the laryngeal area to be protected from infiltration by the bolus.

In summary, the present study demonstrates anatomic and functional innervation of the CPM by the RLN. Gross anatomic dissection revealed that a branch of the RLN enters the muscular wall of the CPM. Selective stimulation of the RLN elicited EMG activity in the CPM, which can only result from neuromuscular connections between the motor nerve fibers of the RLN and CPM fibers. The normal pattern of CPM swallowing EMG activity in sheep is dependent on an intact RLN. Swallow CPM EMG activity elicited by mechanical stimulation of the oropharyngeal area was altered by a shortened duration and loss of CPM EMG large-amplitude activity after section of the RLN. Low-amplitude activity was retained by the CPM during swallowing after RLN section, demonstrating that the CPM has dual innervation from the RLN and from another, as yet unidentified, nerve.

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Received 9 July 1996; accepted in final form 27 February 1997.

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