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CROSS-INNERVATION OF THE THYROARYTENOID MUSCLE BY A BRANCH FROM THE EXTERNAL DIVISION OF THE SUPERIOR LARYNGEAL NERVE

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The neuroanatomy of the larynx was explored in seven dogs to assess whether there is motor innervation to the thyroarytenoid (TA) muscle from the external division of the superior laryngeal nerve (ExSLN). In 3 animals, such innervation was identified. Electrical stimulation of microelectrodes applied to the ExSLN resulted in contraction of the TA muscle, indicating that this nerve is motor in function. This was confirmed by electromyographic recordings from the TA muscle. Videolaryngostroboscopy revealed improvement in vocal fold vibration following stimulation of the ExSLN compared to without it. Previously, the TA muscle was thought to be innervated solely by the recurrent laryngeal nerve. This additional pathway from the ExSLN to the TA muscle may have important clinical implications in the treatment of neurologic laryngeal disorders such as adductor spasmodic dysphonia.

KEY WORDS — adductor spasmodic dysphonia, in vivo canine laryngeal model, laryngeal innervation, recurrent laryngeal nerve, superior laryngeal nerve, thyroarytenoid muscle.

INTRODUCTION

The innervation pattern of the intrinsic laryngeal muscles was initially determined decades ago, with anatomic, histologic, and degenerative studies.[1,2] With the exception of the cricothyroid (CT) muscle, all intrinsic laryngeal muscles are innervated by branches of the recurrent laryngeal nerve (RLN). The CT muscle is innervated by the external branch of the superior laryngeal nerve (ExSLN). Due to limitations of past techniques, the exact branching patterns of small nerves could not be identified. During dissection, it is difficult to distinguish small nerves from blood vessels and connective tissue. Moreover, distal branches are prone to injury during anatomic studies of nerves. Today, more sophisticated histologic methods and microdissection techniques are available to assess the branching patterns of the laryngeal nerves.

The anatomy and physiology of the ExSLN have been controversial. In 1932, Lemere[1] reexamined the innervation pattern of the canine larynx. Anatomically, he discovered that the ExSLN supplied the CT muscle and then pierced the thyroarytenoid (TA) muscle to innervate the mucosa beneath the vocal folds anteriorly. He believed that the ExSLN fibers innervated the TA muscle as they passed through it. Electrical stimulation of the ExSLN did not, however, produce activity in the TA muscle on electromyography (EMG). Lemere was unsuccessful in finding *physiological* evidence of TA innervation by the ExSLN fibers.

In 1993, Sanders et al[3] demonstrated anatomically that the ExSLN may innervate the TA muscle. With Sihler's staining technique, a cross-innervation between RLN terminals and the ExSLN in the TA muscles was found in 4 of 10 excised canine larynges. The ExSLN passed from the posterior surface of the CT muscle across the pyriform sinus and entered the lateral surface of the TA muscle. Since Sihler's method can only provide an anatomic proof of innervation patterns, Sanders et al were unable to determine whether the connection between the ExSLN and the TA muscle contained motor fibers.

The present experiment examined the function and branching pattern of the distal ExSLN in an in vivo canine model. Microanatomic dissection of 7 canine larynges was performed. Electrodes were applied to

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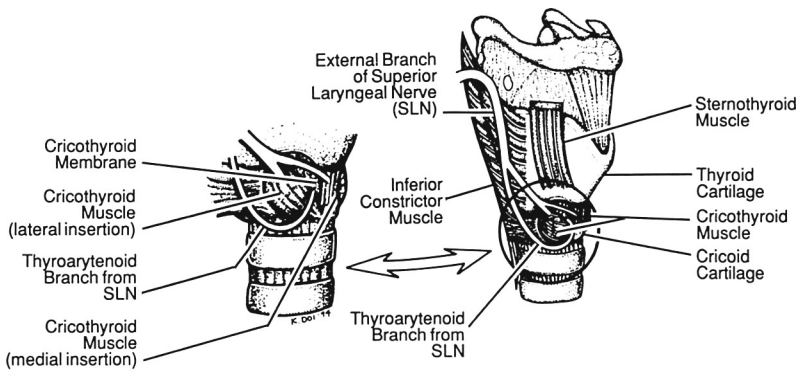

Fig 1. Schematic of thyroarytenoid (TA) branch takeoff from external division of superior laryngeal nerve (ExSLN). Enlarged segment on left side of Figure demonstrates point of entry of TA branch of ExSLN between medial and lateral insertions of cricothyroid muscle. SLN — superior laryngeal nerve.

the dissected nerves in an attempt to produce muscular contraction. The function was confirmed with EMG recordings from the muscles and by videostroboscopic evaluation of the larynges.

MATERIALS AND METHODS

In Vivo Canine Model of Phonation. The in vivo canine model of phonation has been used in our laboratories extensively, and a detailed description has been published.[4] Each animal was initially premedicated with 3 mL of acepromazine maleate intramuscularly. Intravenous pentobarbital sodium (Nembutal) was administered to result in corneal anesthesia. Additional Nembutal was given to maintain this level of anesthesia.

The animal was placed supine on the operating table, and a midline incision was made to expose the trachea from the sternal notch to the hyoid bone. The strap and sternocleidomastoid muscles were retracted laterally to expose the larynx and trachea. A low tracheostomy was performed at the level of the suprasternal notch, through which an endotracheal tube was passed to allow ventilator-assisted respiration. A second tracheostomy was performed in a more cephalad position, through which an endotracheal tube was placed.

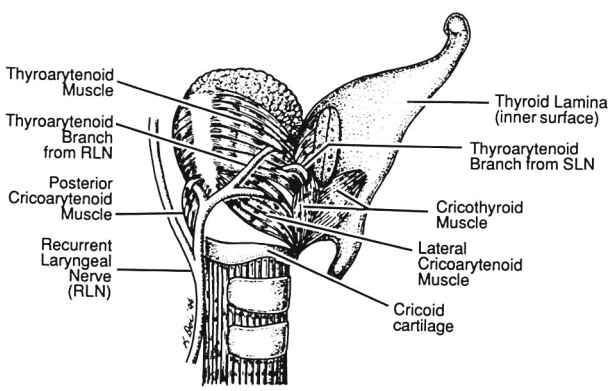

Fig 2. View of intrinsic laryngeal muscles demonstrating TA branches from SLN and recurrent laryngeal nerve (RLN). Thyroid lamina is outfractured to examine muscles present on its inner surface.

The tip of the upper endotracheal tube was positioned approximately 10 cm below the level of the glottis. The cuff was inflated to just seal the trachea. The air was heated and humidified by passing through 5 cm of heated water. It was then passed through the upper endotracheal tube to establish a constant flow system. The temperature of air was 37°C when measured at the glottic outlet. The amount of flow was controlled with a Gilmont flowmeter. Direct visualization of the larynx was possible when the epiglottis was suspended from a fixed point with a 1-cm button.

Bilateral RLNs and ExSLNs were isolated. The RLNs were exposed 5 cm inferior to the larynx in the tracheoesophageal groove; their identity was confirmed with electrical stimulation. A thyroid cartilage window was made to aid in identification of any nerve branches entering or leaving the TA muscle. Under microscopic examination, the TA branch of the RLN was identified as the last terminal branch and its identity was confirmed by electrical stimulation; it was cut distally from the branching point, with care to avoid injury to the other terminal branches.

The ExSLN was isolated at the level of the superior attachment of the sternothyroid muscle to the thyroid cartilage (Fig 1). The edge of the thyroid cartilage was lifted and the pharyngeal constrictor muscle was transected. Next, the CT joint was dislocated and any nerve traveling between the medial and lateral insertions of the CT muscle entering the TA muscle was microdissected (Figs 1 and 2). The nerve was then followed retrograde to verify its origin from the ExSLN.

One electrode was applied to each trunk of the RLN, approximately 5 cm proximal to the takeoff of the posterior division (hereinafter referred to as the "trunk"). The second electrode was applied to the ExSLN nerve branch to the TA muscle. The electrodes were isolated such that they were contacting only the nerve branches and not the muscles. Electrical input to the superior laryngeal nerve (SLN) electrode was supplied by a Grass model 54H stimulator (Grass Instruments, Quincy, Mass). Current stimula-

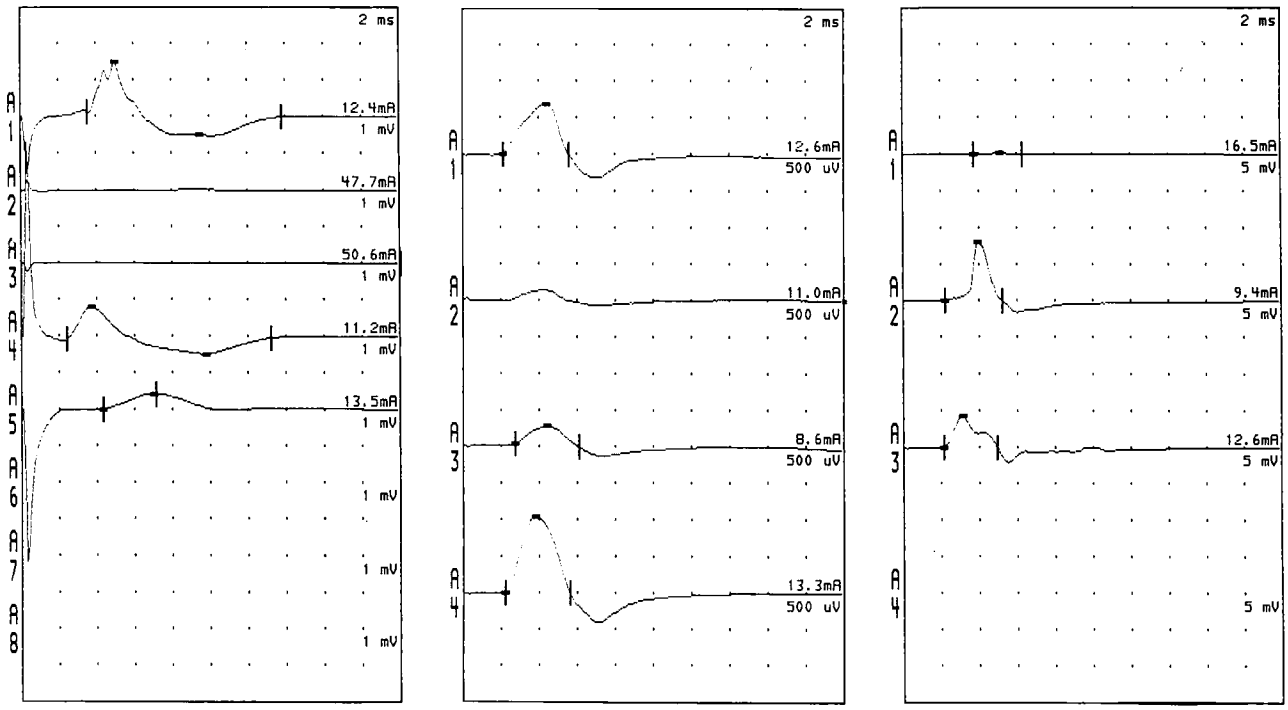

Fig 3. Evoked electromyography tracings from TA muscle. X-axis is time (2 milliseconds per division), and y-axis is amplitude. Intensity of stimulating current is shown in milliamperes. A) Stimulation of TA branch of RLN (A1), RLN "trunk" (A2), internal division of SLN (A3), TA branch of ExSLN (A4), and ExSLN trunk (A5). For amplitude (y-axis), there is 1 mV per division. B) Stimulation of ipsilateral ExSLN trunk (A1) and ipsilateral TA branch of ExSLN (A2 through A4). Recordings were obtained from posterior TA muscle (A2), middle TA muscle (A3), and anterior TA muscle (A4). For amplitude (y-axis), there is 0.5 mV per division. C) Stimulation of contralateral TA branch of ExSLN. Recordings were obtained from posterior TA muscle (A1), anterior TA muscle (A2), and middle TA muscle (A3). For amplitude (y-axis), there are 5 mV per division.

tion to the RLN trunk was provided by a constant current stimulator (WR Medical Electronics RLN Stimulator, model S2LH, St Paul, Minn). The frequency of nerve stimulation was 80 Hz with a pulse duration of 1.5 milliseconds. The intensities used for stimulation ranged from 1.5 to 3.0 V. In order to avoid problems associated with ground loops, the measuring instruments were floated.

Evoked Electromyographic Studies. Evoked EMG was recorded during experimental trials from the TA muscle during stimulation of the ipsilateral and contralateral TA branches of the RLN, as well as the ExSLN trunk and the branch to the TA muscle. The placement was confirmed by stimulating the TA branch of the RLN and recording the EMG signal from the muscle. Stimulation of other branches of the RLN did not result in a recorded EMG signal from the TA muscle. Bipolar concentric EMG needle electrodes were placed transcervically into the TA muscle at the anterior, middle, and posterior thirds of the muscle. The signal was recorded with a Nicolet Viking II EMG instrument. The signal was high-pass-filtered at 20 Hz and low-pass-filtered at 10 kHz. The output was digitized at 20 kHz and stored.

Videolaryngostroboscopy. After the TA branch of the RLN was transected, stroboscopic images of vocal fold movement were obtained during stimulation of the RLN trunk with and without stimulation of the ExSLN. These images were recorded with a Storz laryngostroboscope (model 8000). A Storz 0° telescope was connected to the stroboscope via fluid-filled cables. The image from the telescope was recorded with a Storz CCD (charge-coupled device) video camera (model 9000) and a Sony U-matic videocassette recorder (VO-5800).

Experimental Design. The anatomic microdissection was performed as previously described. Stimulating electrodes were applied to the RLN "trunk" (following transection of the TA branch) and to the main ExSLN, as well as its branch(es) to the TA muscle. First, the TA branches of the ExSLN were stimulated, and any activity of the TA muscle was videorecorded. In addition, EMG recordings were obtained from the muscle. Next, all bellies of the CT muscle were transected, and the videolaryngoscopic and EMG recordings were obtained from the TA muscle during stimulation of the main trunk of the ExSLN. To ensure that this response was not antidromic, the nerve was transected proximal to the site of application of the microelectrode. Finally, the

RLN trunk electrode was stimulated and TA activity was recorded. Videostroboscopy was performed with stimulation of the RLN trunk with or without stimulation of the TA branch of the ExSLN. To avoid volume-conducted potentials, all ExSLN fibers not innervating the TA muscle were transected.

RESULTS

Anatomic Study. Three of 7 animals studied revealed TA branches from the ExSLN. In 2 of the animals, this innervation pattern was bilateral. One of 2 dogs with bilateral TA innervation from the ExSLN had two branches on each side. The other dog had one branch on one side and two branches on the other side. The animal with unilateral innervation had two branches from the ExSLN to the TA muscle.

Physiological Study. Bilateral stimulation of the RLN trunks did not result in phonation. However, in 2 animals with bilateral TA branches from the ExSLN, after simultaneous stimulation of bilateral RLN trunks and TA branches of the ExSLN, phonation ensued, including the presence of mucosal traveling waves. The bulk of the activity of the TA branch of the ExSLN was observed in the anterior third of the vocal fold, although there was some activity posteriorly when the ipsilateral ExSLN TA branch was stimulated. Stimulation of the contralateral TA branch of the ExSLN resulted in slight bulging of the TA muscle only at the anterior third of the muscle.

Electromyographic Study. Figure 3A reveals the EMG recordings from the TA muscle during stimulation of the TA branch from the RLN (A1), the RLN trunk (A2), the internal division of the SLN (A3), the branch from the ExSLN directly to the TA muscle (A4), and the trunk of the ExSLN (A5). The peak-to-peak amplitudes are 2,058, 0, 0, 1,305, and 437.8 μV, respectively. To ensure absence of field artifact and antidromic response, electrodes were isolated from the surrounding tissues. In addition, the experiment was repeated with the contralateral ExSLN transected as well as intact. Finally, the bellies of the CT muscle were transected and the ExSLN was cut proximal to the site of placement of the stimulating electrode.

In Fig 3B, the ExSLN trunk (A1) or the ExSLN branch to the TA muscle (A2 through A4) was stimulated and EMG recordings were obtained from the anterior ipsilateral TA muscle (A1 and A4), the posterior ipsilateral TA muscle (A2), and the mid-ipsilateral TA muscle (A3). The peak-to-peak amplitudes of the EMG signal in A1 through A4 were 701, 111, 245, and 1,057 μV, respectively. The areas under the graphs for the signals were 1,431, 245, 613, and 2,104 μV times milliseconds. It is important to note that simultaneous EMG recordings from other intrinsic laryngeal muscles revealed no electrical activity.

Figure 3C shows the ExSLN branch to the TA muscle being stimulated and the EMG recordings obtained from the anterior (A2), middle (A3), and posterior (A1) parts of the contralateral TA muscle. The EMG recording is strongest anteriorly and becomes weaker as one moves posteriorly.

DISCUSSION

This in vivo study was designed to test the hypothesis, based on previous observations, that motor innervation from the ExSLN to the TA muscle is present in the dog.[3] It has been accepted until recently that the TA, posterior cricoarytenoid, interarytenoid, and lateral cricoarytenoid muscles are innervated solely by the RLN, and that the CT muscle is supplied by the external division of the SLN exclusively.[5,6]

There has been some controversy in the past regarding the pattern of laryngeal innervation. Using dissection and degenerative studies, Exner[7] showed that every laryngeal muscle was innervated bilaterally, by both the SLN and the RLN. New[8] discovered that the internal division of the SLN provides motor innervation to the interarytenoid muscle. Previous studies have relied on anatomic evidence to determine the innervation of the TA muscle.[1,3] Therefore, there is no proof of the motor or sensory nature of this innervation. In this study, anatomic and physiological techniques were used to prove that similar to the TA branch of the RLN, the ExSLN supplies motor innervation to the TA muscle.

In 2 of 7 dogs examined, bilateral innervation from the ExSLN to the TA muscle was present. In 1 animal, this innervation was unilateral. Four dogs did not demonstrate a branch from the ExSLN. Whether this variability represents actual anatomic differences or the difficulty in microdissection of these small nerve branches awaits future investigation. However, the variability found in this study is in agreement with the findings of a previous histologic work.[3]

The EMG signals were recorded in the TA muscle after stimulation of the terminal ExSLN branches to the TA muscle. Evoked EMG is widely used as a tool for determining the function of a muscle in response to stimulation of the motor neurons supplying it.[9,10] The shape and size of the EMG signal can be used to determine the motor nature of the neuronal input.[9] The signals recorded in this study from the TA muscle during stimulation of the ExSLN branches

identify these branches as motor.

The activity of the TA muscle is essential for normal vocal fold vibration and voice production. When activated, this muscle causes a change in vocal fold stiffness and hence the fundamental frequency.[11,12] After the TA branch of the RLN was cut, the RLN "trunk" was stimulated. The resultant phonation was hoarse and the mucosal traveling waves were absent because of the lack of complete closure of the folds. When the RLN trunk was stimulated simultaneously with the bilateral ExSLN branches to the TA muscle, phonation significantly improved. Videolaryngostroboscopy revealed presence of a mucosal traveling wave after stimulation of the bilateral ExSLN branches.

It appears that the bulk of activity of the TA branch of the ExSLN is located in the anterior third of the vocal fold. The EMG recordings showed that as one moved posteriorly along the TA muscle, the peak-to-peak amplitude of the measured signal decreased in size. This may be important in intricate control of the pitch, an important function of the TA muscle.

The fact that the TA has additional motor innervation has many important implications in treating patients with certain voice disorders, such as spasmodic dysphonia. This speech disorder is a localized dystonia, producing hyperadduction of the vocal folds during speech and a halting, strained vocal pattern. The additional innervation may explain why some patients have not had long-term control of adductor spasmodic dysphonia following TA denervation.[13] An attempt will be made in the design of upcoming surgical procedures to identify and section the TA branch from the ExSLN as well as that from the RLN. These patients will then be followed to observe the long-term effect of surgery on control of spasmodic dysphonia.

It should be emphasized that there are some differences between canine and human laryngeal innervation. As an example, in some dogs, there may be another major branch of the RLN, the pararecurrent nerve, that is not observed in humans.[1] Furthermore, both in dogs and in humans, individual variations in the anatomy of the RLN and SLN are fairly common.

In addition to design of new surgical procedures, future studies would include retrograde staining of the TA nerve branches from the RLN and SLN in order to determine their central projection from the nucleus ambiguus. These centers can then be compared to one another and be used to develop a detailed map of the laryngeal motor centers within the nucleus ambiguus. These histologic studies are currently under way in our laboratories.

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