Measurement of Adductory Force of Individual Laryngeal Muscles in an In Vivo Canine Model

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In this experiment, the adductory properties of three intrinsic laryngeal muscles (the thyroarytenoid [TA], lateral cricoarytenoid [LCA], and interarytenoid [IA]) were studied and quantified. Using an in vivo canine laryngeal model, a recently developed "tensionometer" was used to measure the adductory force produced by each of these muscles at the vocal process of the arytenoid. Isolated muscle activation was obtained by stimulating selective terminal branches of the anterior division of the recurrent laryngeal nerve. Results indicate that the LCA is the strongest adductory muscle, followed by the TA and the IA. Videolaryngoscopy revealed that LCA contraction causes adduction of the vocal fold and vocal process, with the predominant effect on the process. TA stimulation leads primarily to adduction of vocal fold, and the IA adducts mainly the vocal process. Implications of these findings are discussed.

INTRODUCTION

Vocal fold adduction is critical for many laryngeal functions, including phonation, effort closure, and closure during swallowing. Adequate airway protection, phylogenetically the most ancient laryngeal function, also requires strong vocal fold adduction. When adductory force is deficient, as in vocal fold paralysis, dysphonia and debilitating aspiration may result.

Knowledge of the adductory force of laryngeal muscles may prove to be important in the development of phonosurgical methods, including laryngeal reconstruction and reinnervation. The literature is ambiguous about the adductory strength of individual laryngeal muscles, largely because objective data are lacking. The relative adductory strength of each intrinsic laryngeal muscle has not been quantified in vivo. Sellars[1] proposed that the most effective adductor of the glottis is the thyroarytenoid (TA) muscle, while Hast and Golbus[2] reported that the lateral cricoarytenoid (LCA) muscle is the primary adductor. Strong and Vaughan[3] concluded that the TA is a tensor/relaxer of the vocal fold that does not contribute significant adductory force. Hirano[4] contends that the combined contractions of the LCA and TA provide the most efficient vocal fold adduction. In addition, he states that the interarytenoid (IA) lacks the strength to fully adduct the vocal fold, instead producing fine adjustments of the glottal aperture during phonation. The IA may also support the LCA and TA during vocal cord adduction, and may stabilize the vocal fold.[4]

Intralaryngeal forces can be measured directly using a force meter (the tensionometer) adapted for use within the larynx. This instrument has been used previously to measure the elastic modulus of the vocal fold in vivo, in both humans and canines.[5–7] In the present experiment, the force rather than the elastic modulus was measured. The goal of this study was to better understand the adductory properties of the LCA, TA, and IA muscles in an in vivo canine model.[8] Individual laryngeal muscles were stimulated, using electrodes applied to the specific branch supplying that muscle, and the tensionometer was used to measure the adductory forces at the vocal process of the arytenoid cartilage. Additionally, videolaryngoscopy was used to describe the specific vocal fold and vocal process movements created by the individual muscle.

MATERIALS AND METHODS

In Vivo Canine Model and Surgical Technique

Three mongrel canines (approximately 25 kg each) were used in this study. Animals were premedicated intramuscularly with acepromazine maleate. Intravenous pentobarbital sodium was administered to a level of corneal anesthesia, and additional doses were given to maintain this level of anesthesia throughout the experiment.

The animal was placed supine on the operating table, and a midline incision was made to visualize the trachea from the hyoid to the sternal notch. The strap muscles and

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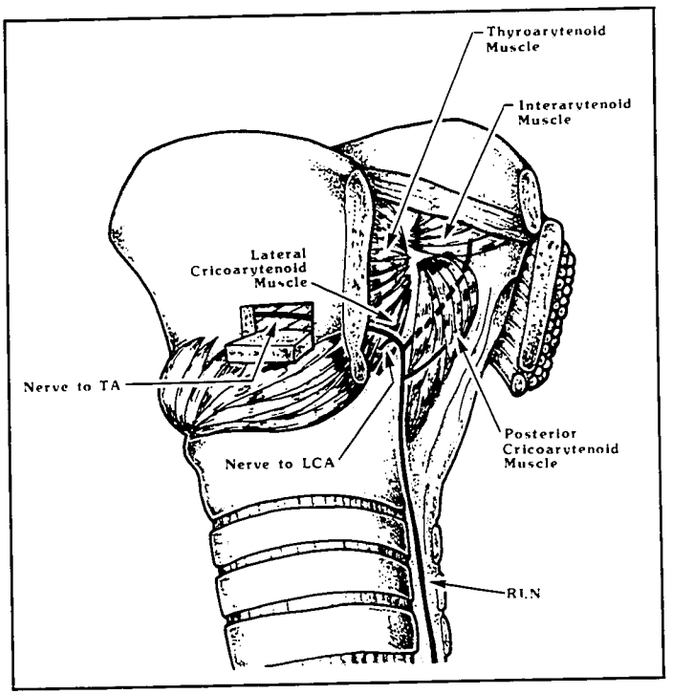

Fig. 1. Intralaryngeal branching of the recurrent laryngeal nerve (RLN) demonstrating a window in the thyroid lamina for access to the thyroarytenoid (TA) branch. (Reprinted with permission from Sercarz, J.A., *et al.*: Bilateral Thyroarytenoid Denervation: A New Treatment for Laryngeal Hyperadduction Disorders Studied in the Canine. *Otolaryngol Head Neck Surg*, 107:657–668, 1992.)

sternocleidomastoid muscle were retracted laterally. A low tracheostomy was performed at the level of the suprasternal notch through which an endotracheal tube was passed to allow ventilator-assisted respiration. The epiglottis was suspended from a fixed point to allow visualization of the anterior larynx.

The superior laryngeal nerve (SLN) and the recurrent laryngeal nerve (RLN) trunks were identified and isolated. The RLN was followed superiorly to the cricothyroid joint adjacent to its entrance into the larynx (Fig. 1). The RLN at this point divides into a small posterior division (PCA branch) directed posteromedially and a larger anterior division that courses anterosuperiorly, supplying the IA, LCA, and TA muscles. The delicate IA branch is located just distal to the posterior division take-off. The distal portion of the anterior division, which branches to the TA, was accessed via an inferiorly based thyroid cartilage flap (Fig. 1).[9]

Rubber electrodes were applied to the RLN branches, as described subsequently. The electrodes were monopolar, contained conductive neopreme with silicone, and were coated with insulative silicone KE45W. The electrodes were attached to a custom-designed, two-channel, constant-voltage DC nerve stimulator. A ground electrode was inserted into the subcutaneous tissue of the neck flap. Nerves were stimulated using 100-Hz pulses with a 1.0-msec duration.

First, the identity of the PCA was verified when brisk abduction occurred with stimulation of the posterior division. The posterior division of the RLN to the PCA was transected. Next, the branch to the IA was identified, and the anterior division was cut distal to that branch. One electrode was applied to the anterior division distal to the IA; this was used to jointly stimulate the TA and the LCA. A second electrode was applied to the main trunk of the RLN; this was used to stimulate the IA. The anterior division of the RLN was transected distal to the LCA branch take-off. An electrode was applied to the TA branch through a window cut in the thyroid cartilage; this electrode was used to stimulate the TA. Finally, the electrode on the anterior division distal to the IA was used to stimulate the LCA.

Measurement of Force

The tensionometer used for force measurement in this study (Fig. 2) was devised to measure the transverse Young's elastic modulus of human vocal folds during laryngeal surgery.[5–7] The elastic modulus is a measure of tissue elasticity, and is proportional to the force required for lateral movement divided by the degree of displacement of the fold (Young's modulus = stress/strain). The same instrument can be used to quantify the adductory force produced at the vocal process. It is able to measure force rapidly, thus minimizing the effect of strain "creep" and stress "relaxation" in vocal fold tissue.[10] In addition, the instrument is accurate to 0.1-g resolution, allowing detection of small differences between the muscles and measurement of the potentially weak IA adduction.

The footplate of the tensionometer was placed in contact with the medial margin of the vocal process of the arytenoid in each hemilarynx. Coarse, intermediate, and

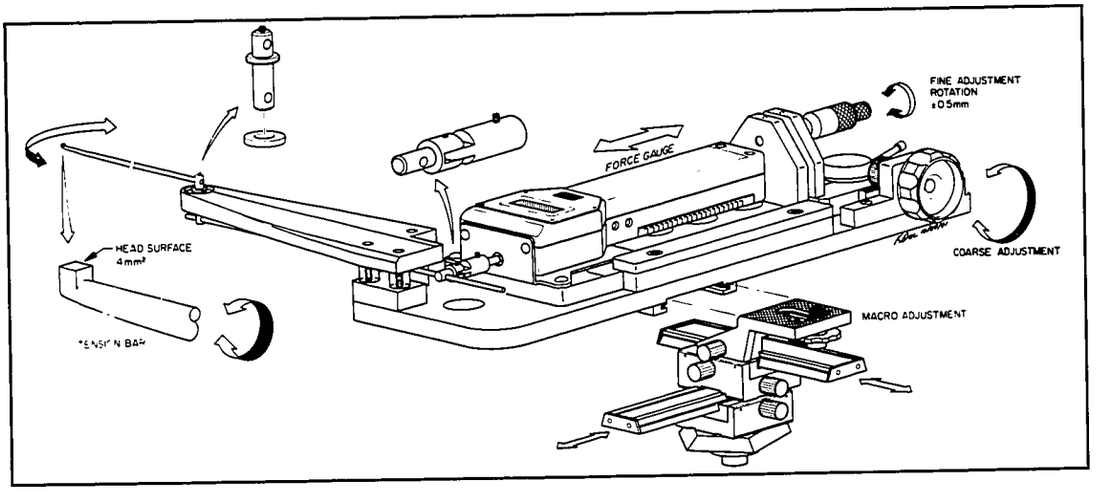

Fig. 2. Schematic diagram of tensionometer used for intralaryngeal force measurement. (Reprinted with permission from Tran, Q.T., *et al.*: Measurement of Young's Modulus in the In Vivo Human Vocal Folds. *Ann Otol Rhinol Laryngol*, 102:584–591, 1993.)

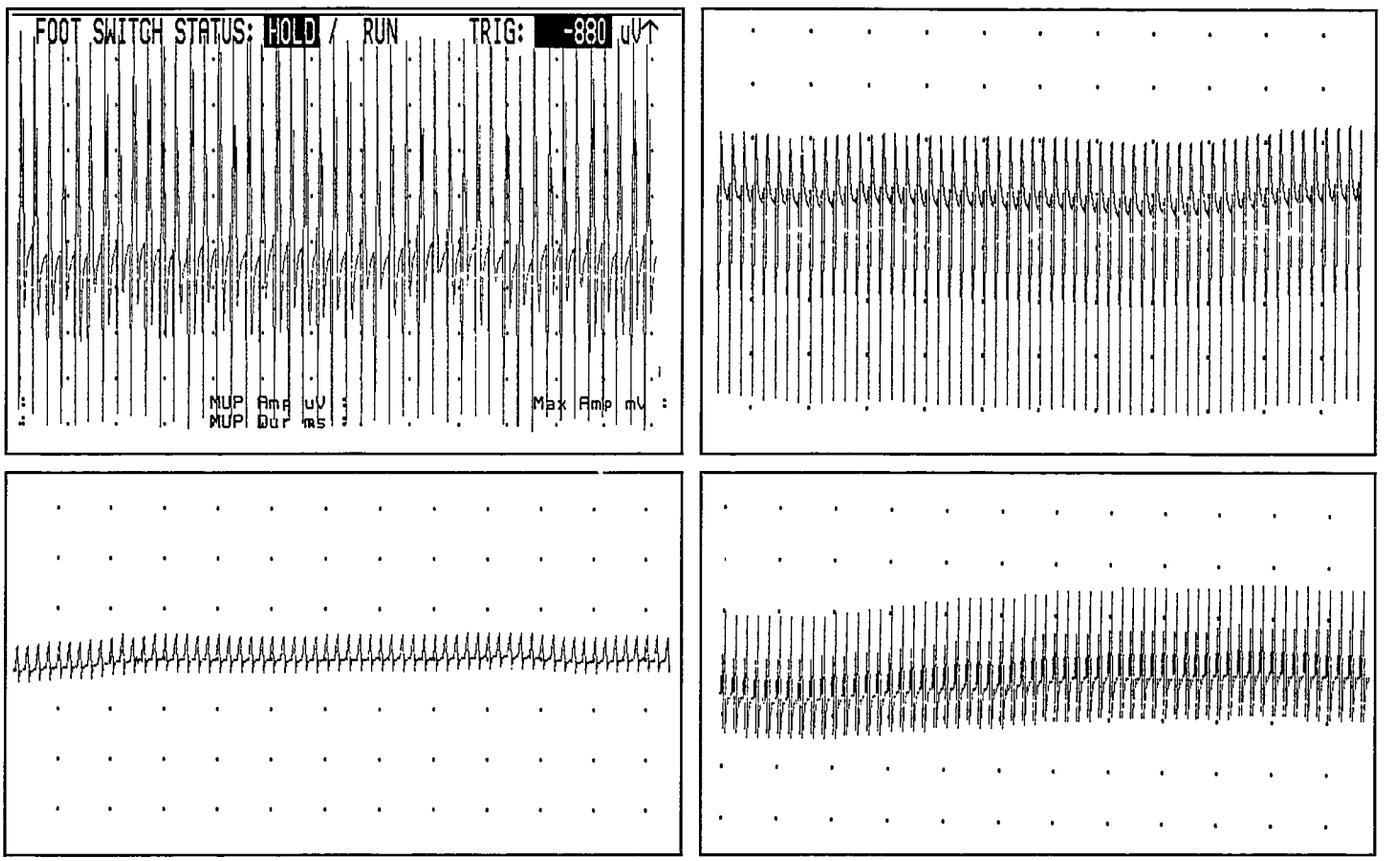

Fig. 3. Evoked electromyographic (EMG) tracings from individual muscles. **Top left.** Evoked EMG tracing from posterior cricoarytenoid (PCA) during stimulation of the posterior division of the RLN. The *x*-axis is time (50 msec/division); the *y*-axis is amplitude (500 μV/division). **Top right.** Evoked EMG tracing from TA during stimulation of the TA nerve branch through the window in the thyroid lamina. The *x*-axis is time (100 msec/division); the *y*-axis is amplitude (500 μV/division). **Bottom left.** Evoked EMG tracing from interarytenoid (IA) during stimulation of its nerve branch from the anterior division of the RLN. The *x*-axis is time (100 msec/division); the *y*-axis is amplitude (500 μV/division). **Bottom right.** Evoked EMG tracing from lateral cricoarytenoid (LCA) during stimulation of its RLN nerve branch. The *x*-axis is time (100 msec/division); the y-axis is amplitude (1 mV/division).

micrometer adjustments allow footplate position to be adjusted by as little as 0.1 mm. Force was measured using a Shimpo Digital Force Gauge (DF-0.5R, Shimpo American Corp.), which combines a light-beam detection design with a microcomputer for measurement of push-pull forces. The force gauge is attached to the deflection bar through a mechanical linkage that allows an exact transition of force (with minimum loss) between the footplate and gauge. The force was measured by positioning the force transducer on the vocal process with the larynx at rest and setting the transducer on zero for each trial. The force was then measured during electrical stimulation of the nerve in question. Force per unit area was calculated by dividing the measured force by the area of the footplate (0.04 cm^2). The stiffness of the deflection bar is included in the calibration of the instrument.

Experimental Design

Six complete sets of measurements were gathered from three canines, except that one dog could be phonated only at two levels of nerve stimulation. The order of muscles studied corresponded to the order of nerve transection described above. The branch to the combined TA and LCA was stimulated first, followed by the IA, the TA, and the LCA. The lowest level of nerve stimulation that produced an adductory force was chosen as low stimulation. The high level of stimulation was the minimum current necessary to produce the maximum measured adductory force. The medium level was the mean of the low and high stimulation current. Stimulation frequency was 100 Hz, at 1.0-msec intervals. Five trials were obtained for each level of stimulation; trials were separated by 3 to 5 minutes to reduce fatigue effects.

During each trial in the same hemilarynx, the same level of nerve stimulation was used for each nerve branch. This method produced the most realistic estimation of comparative force in the awake animal, since presumably equal levels of current are distributed to each nerve branch in the RLN in the natural state.

Evoked Electromyography

Evoked electromyography (EMG) was recorded during experimental trials for one of the mongrel dogs for each nerve branch and associated muscle, to confirm presence or absence of muscular activity in stimulated muscles. Concentric EMG needle electrodes were placed transorally into the TA, IA, and LCA muscles. The signals were recorded using 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.

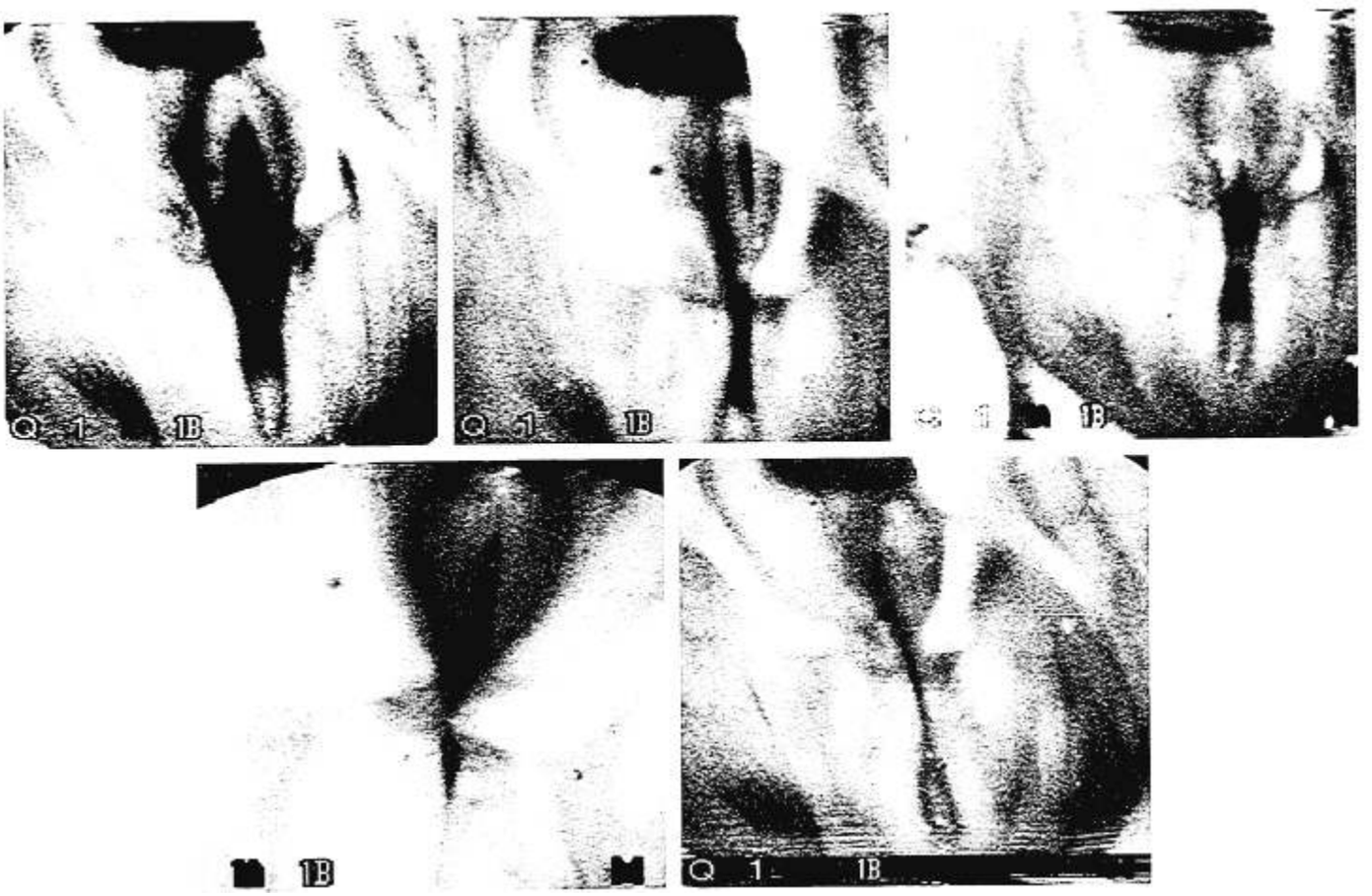

Fig. 4. Videolaryngoscopy of the canine larynx in the following conditions: **Top left.** Resting state without nerve stimulation. **Top center.** Bilateral stimulation of the LCA nerve branch. **Top right.** Bilateral stimulation of the TA nerve branch. **Bottom left.** Bilateral stimulation of the IA nerve branch. **Bottom right.** Bilateral simultaneous stimulation of the TA and LCA branches.

Videolaryngoscopy

Videolaryngoscopy was performed with a Karl Storz (Culver City, Calif.) 0-degree endoscope connected to a halogen light source via a fluid-filled light cable. Images were recorded with a Jedmed CCD (charge-coupled device) video camera (model 70-5110) and a Sony U-matic videotape recorder (VO-91850). This system allowed a frame-by-frame analysis of the video images.

RESULTS

Evoked Electromyography

Evoked EMG recordings are shown in Figure 3. The evoked EMG of the PCA muscle during stimulation of the posterior division of the RLN is shown. Normal muscle activity is present, but disappears after transection of this nerve. This ensures that pure adductory forces were measured, without any contamination from the PCA muscle, the sole abductor of the vocal fold.[11,12]

EMG tracings from the TA, IA, and LCA measured in one canine are also shown in Figure 3. The tracings were recorded while specific branches to these muscles were stimulated, and thus represent exclusive activity of each muscle. The concentric EMG needle was placed in different parts of the same muscle during the stimulation of its nerve branch to assure activity of all parts of the muscle. The mean peak-to-peak values of 10 PCA, LCA, TA, and IA EMG tracings were 1.75 mV, 1.0 mV, 0.85 mV, and 0.125 mV, respectively. EMG activity was not present in any muscle other than the one corresponding to the specific nerve branch being stimulated.

Videolaryngoscopy

Figure 4 shows videolaryngoscopy frames obtained using a Sony 5000 color video printer. In Figure 4 (top left), no nerve was stimulated and the glottis is open. In Figure 4 (top center), both LCA branches were stimulated. Both vocal processes and vocal folds are adducted, with predominant effect on the vocal process and a moderate-sized midfold gap. In Figure 4 (top right), both TA branches were stimulated. The folds are adducted, with less effect on the vocal process and a posterior glottic gap. When both IA branches are stimulated (bottom left), the vocal processes are adducted, with minimal effect on the folds. Figure 4 (bottom right) shows stimulation of LCA and

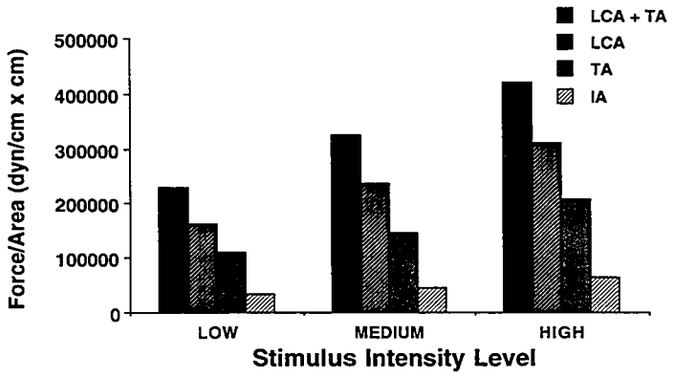

Fig. 5. Adductory forces generated by LCA, TA, IA, and combined TA/LCA stimulation at the three levels of current.

TA. The glottis is completely closed anteriorly and posteriorly, without any noticeable gaps.

Measures of Adductory Force

Adductory force/area generated by the TA, IA, LCA, and the combined TA/LCA for two dogs is shown in Figure 5. Identical results were obtained for a third dog, but these were not analyzed statistically because of differences in the stimulation conditions, as described previously. Force per unit was calculated by dividing the force measured by the tensionometer by the area of the footplate in contact with the vocal process. The area of the footplate was measured to be exactly 0.04 cm^2. A four-way repeated measures analysis of variance (dog by hemilarynx by stimulation level by muscle, with repeated measures for muscle) showed that muscles differed significantly in their adductory force/area ($F(3,144) = 12617.20$, $P<.01$). Stimulation levels also differed significantly ($F(2,48) = 3979.74$, $P<.01$). Significant differences among dogs occurred in the overall level of adductory force/area generated ($F(1,48) = 21699.81$, $P<.01$), but differences between hemilarynges and all interaction terms were negligible, each accounting for less than 1% of variance in the underlying data.

These findings suggest that, although dogs differed in the overall magnitude of forces generated in this experiment, the specific patterns of muscular response to stimulation were comparable across subjects. Planned comparisons showed that the force generated by the LCA was significantly greater than that of the TA ($F(12,48) = 259.71$, $P<.01$). The TA, in turn, was significantly stronger than the IA ($F(12,48) = 1057.91$, $P<.01$). Finally, the combined TA/LCA muscles were significantly stronger than the LCA alone ($F(12,48) = 254.22$, $P<.01$). An identical pattern of muscular response was also found for the partial data set obtained from dog 3.

DISCUSSION

This study examined the quality and quantity of the adductory force of the intrinsic laryngeal muscles. Results indicate that the LCA produces the strongest adductory force among the intrinsic laryngeal muscles, and causes both vocal fold and vocal process adduction. Contraction of the TA, the second strongest adductor, results primarily in adduction of the mid–vocal fold, with a lesser effect on the vocal process. The IA is the weakest vocal fold adductor, causing adduction solely at the vocal process.

In order to ensure that nerve stimulation produced contraction of the intended muscles, evoked EMG recordings were performed simultaneously with branch stimulation.[13] EMG activity was not present in muscles that were not stimulated. In addition, EMG recordings confirmed that no activity remained in the PCA following transection of the posterior division of RLN. The function and action of the laryngeal muscles depend on the position of the vocal fold and process. In order to isolate the action of each muscle, the nerve branches were isolated and stimulated individually to avoid functional interference by the other adductors. This approach avoids direct stimulation of muscles, which could have resulted in spreading of stimulation to neighboring muscles. Additionally, several laryngeal muscles (*e.g.*, TA, PCA, LCA) have distinct neuromuscular bellies. Direct stimulation might have activated only one of the bellies.[11,14,15] The posterior division of the RLN was also transected to abolish the relatively pure abduction produced by the PCA muscle.[11,12]

In addition to independent stimulation of each adductor, the anterior branch of the RLN distal to the division of the IA branch was stimulated to study the additive effects of adductory forces in the LCA and TA. Results indicate that the adductory force generated simultaneously by the two muscles peaked at about 130% of the force of the LCA.

One limitation of this study is that the measurement of force was performed only in the horizontal plane. However, this component of force is most important in several of the functions of the larynx, including force closure and phonatory control. Baken and Isshiki[16] showed that LCA contraction in an excised human larynx resulted in adduction and inferior projection of the vocal process. Hirano, *et al.*[17] stated that, in an excised human larynx, LCA/TA contraction causes medial and dorsal displacement of the tip of the vocal process. Vectors of force are also important in understanding the physiology of vocal fold movement, because each muscle may have a diverse group of fibers with different force vectors.[1,11,17] For example, the PCA has three distinct neuromuscular bellies with separate vectors of pull on the vocal process,[12] and the TA has three directions of forces acting on the arytenoid resulting from three muscle fiber directions.[1] Thus the precise anatomy of each muscle can be important in understanding its specific function and action. Because the tensionometer was used to measure only the horizontal force vector of each muscle, this study cannot determine the specific directions of the force generated.

The vocal process of the arytenoid was chosen as the site for measurement of adductory forces for several reasons. Most importantly, all three muscles studied insert onto the arytenoid and, as a result, exert their immediate effect on this cartilage. The vocal process of the arytenoid cartilage has its tip positioned at the posterior end of the vocal fold because of its attachment to the vocal ligament. The motion of the vocal process would therefore be related to the adductory and abductory motion of the vocal fold. Baken and Isshiki[16] previously used the vocal process in their study of intrinsic laryngeal muscles, and argue that the position of the vocal process provides an accurate representation of the adductory force exerted on the arytenoid cartilage. Finally, the vocal process allows easy access for placement of the tensionometer footplate, resulting in more consistent recordings and an accurate reference point for future experiments.

Videolaryngoscopy was used to describe vocal fold and vocal process movements created by the adductory muscles. The LCA adducts both the vocal process and fold with the prevailing effect on the vocal process, leaving a midline gap in the fold. The TA adducts primarily the vocal fold, with a lesser effect on the vocal process. The IA predominantly adducts the vocal process; its function appears to be accessory to that of the LCA and TA. The IA may be necessary to completely close the posterior glottal chink. Video images further support the claim that the TA is not exclusively a tensor/relaxer muscle of the larynx, but is also an important adductor of both the vocal fold and process. Although the TA primarily adducts the vocal fold, its adductory force on the vocal process is greater than that of the IA. The results of the videolaryngoscopy closely resemble the findings of Hirano and his colleagues in their study of excised canine larynges.[4]

The biomechanical properties of the larynx, such as adductory force studied in this experiment, have potential clinical relevance. For example, one goal of laryngeal reinnervation may be to medialize the mid–vocal fold. This would be desired in a relatively medialized vocal fold in a patient with mid-glottal gap, marked by abnormally low laryngeal resistance during phonation and a breathy voice. In this setting, selective reinnervation of the TA would likely produce the desired mid–fold closure and increase vocal efficiency. By understanding how each muscle contributes to vocal fold adduction, strategies of selective reinnervation may be tailored in the future for the treatment of vocal fold paralysis. This work may also have relevance for the treatment of spasmodic dysphonia, in which forceful hyperadduction of the vocal folds produces unwanted phonatory effects. Selective denervation of specific nerve branches may be used in future attempts to treat laryngeal hyperadduction disorders.[9]

CONCLUSION

This study has shown that LCA is the strongest adductor among the intrinsic laryngeal muscles, followed by TA and IA. Furthermore, videolaryngoscopy has clarified the adductory functions of these muscles: LCA adducts the vocal process and vocal fold with the predominant effect on the processes leaving a midline gap in the folds; TA adducts the vocal fold primarily with less effect on the processes; IA essentially adducts the vocal process with minimal effect on the vocal fold; and TA, in conjunction with LCA, completely closes the glottis. These findings may have important clinical applications in treatment of patients with laryngeal insufficiency or hyperadduction.

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