Mucosal afferents mediate laryngeal adductor responses in the cat

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Received 13 May 2002; accepted in final form 17 July 2002

Andreatta, Richard D., Eric A. Mann, Christopher J. Poletto, and Christy L. Ludlow. Mucosal afferents mediate laryngeal adductor responses in the cat. J Appl Physiol 93: 1622–1629, 2002. First published July 19, 2002; 10.1152/japplphysiol.00417.2002.—Laryngeal adductor responses (LAR) close the airway in response to stimulation of peripheral afferents in the superior laryngeal nerve. Although both mucosal afferents and proprioceptive receptors are present in the larynx, their relative contribution for reflex elicitation is unknown. Our purpose was to determine which receptor types are of importance in eliciting the LAR. A servomotor with displacement feedback was used to deliver punctate displacements to the body of the arytenoid cartilage and overlying mucosa on each side of the larynx in eight anesthetized cats. The same displacements were delivered both before and after surgical excision of the overlying mucosa. With the mucosa intact, early short-latency component R1 LAR responses recorded from the thyroarytenoid muscles were frequent (ipsilateral > 92%, contralateral > 95%). After the mucosa was removed, the LAR became infrequent (<3%) and was reduced in amplitude in both the ipsilateral and contralateral thyroarytenoid muscle recording sites (P < 0.0005). These findings demonstrate that mucosal mechanoreceptors and not proprioceptive afferents contribute to the elicitation of LAR responses in the cat.

sensorimotor; electromyogram; servomotor displacement; thyroarytenoid

Peripheral afferents in the mammalian larynx provide a variety of physiological inputs through specialized end organs, including pneumoreceptor, chemoreceptor, thermoreceptor, proprioceptive, and mechanoreceptive afferents (14, 31–33, 35, 51–53). These different afferent populations are contained in the internal branch of the superior laryngeal nerve (ISLN) and are thought to modulate activity in the laryngeal motoneuron pools during cough, swallow, and vocalization (4, 44, 49). Although extensive research has been conducted on the role of afferents contained in the mucosa on laryngeal control, less is known about the role of proprioceptive joint and muscle afferents on laryngeal protective reflexes.

Mechanical indentations or vibratory stimulation to the mucosa of the cat larynx have been demonstrated to elicit responses in superior laryngeal nerve fibers (8, 9). Similar investigations have confirmed that the activity in these receptors is well correlated with light touch, air pressure changes, laryngeal muscle contraction, and cartilage displacement in animal models (6, 13, 26, 34) and are in evidence in the human (2). Davis and Nail (8) found that the glottis is densely populated with low-threshold, rapidly adapting (RA) mechanoreceptors of small receptive field size. The largest proportion of these RA afferents were in the region surrounding the arytenoid cartilage in the cat (8, 21). The arytenoid cartilages adjust their position to produce vocal fold closure during cough, swallow, and vocalization. Recordings have demonstrated that these receptors provide movement-related afferent feedback during evoked vocalization in the cat (44), are contained in the ISLN, and contribute to muscle activity during vocalization.

The laryngeal adductor response (LAR) is a rapid burst of activity in the thyroarytenoid (TA) muscles, which can assist in closure of the larynx in response to ISLN stimulation. The spatiotemporal properties of the LAR have been studied in both humans and animals by using a variety of stimulus conditions (17, 37, 40, 50). Besides the well-known role of the LAR for airway closure (37), it has been suggested that this sensorimotor pathway is also involved in regulating the stiffness of the internal laryngeal musculature during vocalization in mammals (1, 10, 48, 49). The LAR pathway is believed to be mediated by afferents from neurons within the nodose ganglion that project to the interstitial subnucleus of the tractus solitarius (11). Interneuronal projections may pass through the lateral tegmental field to the laryngeal motor neurons in the nucleus ambiguus (5, 7, 44).

The purpose of this study was to determine the relative contribution of mucosal mechanoreceptor afferents and cricoarytenoid joint proprioceptors in the elicitation of the short-latency component of the LAR. A surgically performed mucosal peel was used to assess the relative contribution of surface mechanoreceptors vs. deep proprioceptive receptors in eliciting LAR re-
sponses in the anesthetized cat. Mucosal excision was used because of possible variations in the degree of tissue diffusion and the duration of effect when applying lidocaine to the mucosa. Access to the vocal folds and the arytenoid region was possible via a carefully performed midline anterior incision through the thyroid and cricoid cartilages. Because the recurrent laryngeal nerve (RLN), which innervates the laryngeal adductor muscles, enters the larynx posteriorly and from an inferior direction, the anterior midline incision of the thyroid cartilage did not interfere with the course of the RLN innervating the TA muscle. Demonstration that the RLN was intact, as evidenced by TA muscle LAR responses, was required before beginning the study. In addition, it was assumed that because the afferents from the region beneath the vocal folds, which join the recurrent nerve, do so in the posterior direction, the anterior midline section through the thyroid cartilage was unlikely. This approach for accessing the vocal folds also allowed us to study the spatial distribution and displacement characteristics of stimulation that evoked the LAR.

METHODS

Subjects and surgical procedures. Eight cats of either sex were used (weight range: 2.6–4.4 kg). All care and treatment procedures were in compliance with and in accordance to the rules and regulations of the National Institute of Neurological Disorders and Stroke Intramural Program (National Institutes of Health Manual 3040-2, revised 1999). Before study, each animal had a cephalic vein port placed in the forelimb for intravenous administration and was preanesthetized with acepromazine (0.1 mg/kg IM). Deep anesthesia was induced with a mixture of 3–5% isoflurane and 100% oxygen (3 l/min) delivered via a ventilator into an enclosed induction chamber. After anesthesia to effect, the animal was removed from the induction chamber, laid supine on the surgical carriage, and fitted with a nose cone to support ventilation with 0.5–1% isoflurane (2 l/min of oxygen), and the skull was fixed to a stereotaxic frame with ear bars.

A tracheotomy was performed at a level superior to the thoracic inlet to provide an independent airway and to allow for discrete stimulation to the laryngeal area without producing airway interruption or stimulating pressure receptors within the lungs. After placement of the endotracheal cannula, α-chloralose (40 mg/kg) was administered intravenously to effect during gradual withdrawal of isoflurane to engender long-lasting anesthesia. Spontaneous breathing was supported throughout the remainder of the protocol via the endotracheal cannula (100% oxygen at 2 l/min). Core body temperature was monitored and maintained between 36 and 38°C by using a warm-water circulating blanket. Vital signs, including heart rate, respiratory rate, PCO2, oxygen saturation, and checks for the lack of a withdrawal response to painful stimuli, were recorded every 30 min. A pediatric-size adhesive grounding electrode was affixed to the shaved dorsal spine region of the animal.

To expose the laryngeal region for mechanical stimulation, a midline separation of the thyroid and cricoid lamina was performed. The free ends of the divided cartilaginous tissue were sutured and secured to the stereotaxic frame to maintain the larynx in a constant open position. Bipolar stainless steel bifilar hooked-wire electrodes contained in a 27-gauge hypodermic needle were inserted into the anterior portion of the TA muscles bilaterally under visual guidance. The lower abdomen was opened to visualize the inferior surface of the diaphragm, and a bipolar hooked wire electrode was inserted. The electromyographic (EMG) signal of the diaphragm was routed to an audio speaker and used to time the delivery of the mechanical stimulus during the expiratory phase of respiration to control for respiratory modulation of the LAR. The exposed mucosa was kept moist by administration of physiological saline periodically throughout the experiment.

Mechanical stimuli. A custom-designed servomotor operating under displacement feedback was used to deliver punctate mechanical transients to three different locations along the exposed vocal fold margin: 1) the tissues encompassing the body of the arytenoid cartilage, 2) the area of the vocal process, and 3) the anterior aspect of the TA muscle (Fig. 1). A 5-mA Transistor-Transistor Logic pulse produced by a Master 8 pulse generator provided the control signal to the servomotor’s controller. The stimulator system consisted of a high-performance audio speaker with a permanently affixed and rigid shaft extending from the speaker’s diaphragm. Displacement of the shaft was monitored by an optoelectrical sensor located distal to the motor and a servo-feedback circuit to the speaker driver. The distal end of the rigid shaft was fitted with a miniature load cell (Schaevitz Sensors, Hampton, VA), serially coupled to a probe tip. The probe tip consisted of a 30-gauge hypodermic needle hub with its cannula trimmed to &lt;2 mm in length. The trimmed cannula was inserted into the mucosa and operated to anchor the probe tip securely in place at each stimulus site. After cannula insertion, the probe was carefully advanced with the use
of a micrometer until the flat and blunt surface of the hypodermic’s plastic hub contacted the tissue surface. During placement of the probe tip over the arytenoid, the 2-mm-trimmed cannula was inserted through the mucosa and anchored into the body of the arytenoid cartilage. The probe tip’s loading was monitored and controlled throughout the experiment for all stimulation sites (preload bias of 30–40 g for all experimental conditions). The direction of stimulation was generally posterolateral.

During placement of the stimulation probe, care was taken to assure that the probe tip was not over the region of the hooked wire electrodes within the TA muscle. This was of particular concern during stimulation of the anterior region of the TA muscle site. The EMG signal was reviewed during the experiment to ensure that movement artifacts were not observed, such as a direct current offset during probe displacement, which might alter the measurement of a muscle response. TA muscle EMG signals were band-pass filtered (30–3,000 Hz) (Fig. 2). After antialiasing with a low-pass filter at 2,500 Hz, stimulus-triggered digitization of the TA EMG and the servo displacement signals were conducted at 5,000 Hz by using a customized routine written in LABVIEW command language (National Instruments).

Experimental conditions. The effects of three different experimental conditions on the ipsilateral and contralateral TA response were studied: 1) a stimulation site condition comparing evoked responses to the same displacement over the arytenoid body, the vocal process, and the TA muscle (on the same vocal fold margin); 2) a displacement magnitude condition comparing evoked responses to different mucosal displacements over the right and/or left arytenoid body, and, lastly; 3) a mucosal peel condition that sought to compare LAR responses to the same displacement of the arytenoid body with and without the overlying mucosa intact. Six of the eight animals received each of the three experimental conditions (Table 1). The remaining two animals received only one or two of the three experimental conditions. For the stimulation site condition, all three loci were on the same side of the larynx. For the displacement magnitude and mucosal peel conditions, results of displacement of an arytenoid cartilage were compared on one side of the larynx. Given the delicate nature and sensitivity of vocal fold tissues to external manipulations, the order of data acquisition was designed to maximize data collection and minimize physical damage at each stimulus site. Therefore, the site condition was typically performed first on one side, followed by the displacement magnitude condition on the opposite side, with the mucosal peel condition performed on the same side as the displacement magnitude condition. For several of the animals, we were able to complete a second set of displacement magnitude and mucosal peel experiments on the remaining intact arytenoid body after completion of the former two experimental conditions (Table 1).

For the site condition, the servomotor was positioned on each of the stimulation sites in a random order. The servo device was programmed to deliver a constant displacement of ~350–400 μm with a loading bias maintained between ~30 and 40 g at each site. Ten tokens were delivered, with 1-min intervals between tokens, at each stimulus site. For the displacement magnitude condition, the servomotor was positioned on the body of the arytenoid cartilage and stimulated

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<th>Stimulus Magnitude Study</th>
<th>Mucosal Peel Study</th>
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Procedure totals n = 6  n = 9  n = 13

ary, Arytenoid cartilage; L, left side; R, right side.
at three discrete displacement levels: ~400, ~250, and ~150 μm (±25 μm for each displacement level). The order of displacement level presentation was randomized with 10 tokens, presented at 1-min intervals, for each displacement magnitude. Lastly, for the mucosal peel condition, a series of 10 tokens at ~325 μm displacement were delivered to the body of the arytenoid cartilage (prepeel condition) at 1-min intervals before surgical removal of the mucosa. The probe tip was then retracted. The peel procedure consisted of undermining and excising the mucosa overlying the arytenoid cartilage with microdissection instruments under high-power magnification. Care was taken to selectively excise the soft tissue and maintain minimal bleeding in the region. After removal of the mucosa, the bare surface of the arytenoid cartilage could be seen, and the probe tip of the servomotor was subsequently repositioned in the exact same position by using the previous tip insertion puncture in the arytenoid cartilage as a location marker. The area was mechanically restimulated at the same displacement level and by using the same preload as in the prepeel condition to assess the effect of mucosal removal on the LAR. On completion of the study, the animals were euthanized by a barbiturate overdose.

Data analysis. Digitized signals were visually inspected and marked by use of a customized routine written in MATLAB (v 5.3). The servomotor displacement signal was used to synchronize the graphical display to aid in identification of the evoked TA responses, bilaterally (Fig. 2). Onset and offset points were marked on the data records and were saved to an ASCII output file. The mean level of rectified TA EMG activity during a 20-ms period before stimulus onset was used to compute and subtract the baseline activity from the response. The initial deflection of the TA EMG response from baseline was identified as the onset of the short-latency component of the TA response, referred to as R1 (~17 ms after the servomotor displacement signal). Response offset was marked as the point when the EMG signal returned to baseline and remained stable for at least 20 ms. Automated signal-processing routines were later used to compute various measures from each marked R1 component of the TA signal, including 1) response latency, 2) response duration, and 3) the total area under the curve (mV × ms), as a measure of response magnitude. The mean baseline was multiplied by the R1 duration and subtracted from the total R1 integral to correct for differences in overall muscle activity independent from the response.

Repeated-measures ANOVAs (α = 0.05) were conducted separately for the site and growth curve conditions using the calculated R1 integral values as the dependent measure. For the site condition, the main effects of stimulus location and side of response were tested along with the interaction of location and side. For the growth curve condition, the main effects of stimulus magnitude and side of response were tested along with the interaction of location and side. Finally, for the mucosal peel condition, mean response rates were calculated by dividing the total number of evoked TA responses by the total number of tokens presented for each animal in the pre- and postpeel condition. Any EMG activity beyond a latency of ~10 ms that differed from the pretrigger baseline and that had a clear onset/offset location was measured and counted as an evoked TA response. The frequencies of LAR responses were compared using a repeated-measures ANOVA. For this analysis, the main effects of peel condition (pre- vs. postpeel) and side of response were tested along with the interaction effect of peel condition and side. Post hoc testing used Tukey’s test for simultaneous pairwise comparisons to examine any omnibus significant main effects after each of the repeated-measures analyses.

RESULTS

Site of stimulation effects. As shown in Fig. 3, the magnitude of the LAR evoked by a constant servomotor displacement of ~350–400 μm increased significantly as mechanical inputs were delivered closer to the body of the arytenoid cartilage for both ipsilateral and contralateral recording sites ($F = 62.96$, $P < 0.0005$). The main effect of response side (ipsi- vs. contralateral) was nonsignificant ($F = 3.09$, $P = 0.139$), as was the interaction effect for site of stimulation by response side ($F = 2.03$, $P = 0.183$). Post hoc testing for the significant main effect of stimulation site revealed that the pairwise comparisons of arytenoid body stimulation with vocal process stimulation and with TA muscle stimulation were significantly different ($P = 0.0024$ and $P = 0.004$, respectively). The pairwise comparison between vocal process stimulation and TA muscle stimulation was nonsignificant ($P = 0.74$).
Stimulation magnitude effects. As expected, the magnitude of the evoked LAR changed systematically as a function of the displacement level delivered to the body of the arytenoid cartilage ($F = 26.91, P < 0.0005$) (Fig. 4). The main effect for stimulation magnitude was significant ($F = 26.91, P < 0.0005$). The main effect for response side (ipsi- vs. contralateral) was nonsignificant ($F = 2.96, P = 0.123$), as was the interaction effect for stimulation magnitude by response side ($F = 1.77, P = 0.203$). Post hoc testing for the significant main effect of stimulation magnitude revealed that the comparison between the highest displacement magnitude vs. the lowest displacement level was significant ($P < 0.0005$) and therefore accounted for the significant omnibus $F$ value. Pairwise comparison of the highest displacement magnitude with the middle level was nonsignificant ($P = 0.106$), as was the comparison between the middle displacement magnitude vs. the lowest magnitude ($P = 0.1082$).

Mucosal peel effects. Stimulation of the arytenoid mucosa during the prepeel condition with a 325-μm displacement consistently evoked the short-latency component of the LAR, bilaterally. The mean percent response rate (pooled across all animals and response sites) was 93.7% (225 responses out of 240 tokens). In contrast, after the mucosa was surgically removed, the LAR mean percent response rate dropped to 1.2%, bilaterally (3 responses out of 237 tokens). A repeated-measures ANOVA ($\alpha = 0.05$) comparing the mean response frequency for each animal pre- and postpeel on each side was significant ($F = 713.36, P < 0.0005$). The pronounced effect of the mucosal peel condition can be clearly seen in Fig. 5, bilaterally. The main effect for response side was nonsignificant ($F = 0.01, P = 0.922$), as was the response side by peel condition interaction ($F = 0.68, P = 0.443$).

DISCUSSION

The most important finding in this experiment was that surgical removal of the mucosa overlying the arytenoid body effectively abolished the LAR response in both the ipsilateral and contralateral TA muscles in the cat. The mean percent response rate dropped dramatically immediately after the mucosal peel, and the area was restimulated with the same displacement

Fig. 4. Mean integrated EMG responses at 3 displacements magnitudes: low (150 μm), middle (250 μm), and high (400 μm). Each point is the average of 10 trials for a single animal at each displacement. Lines connect responses within the same animal. A: ipsilateral TA muscle response amplitudes. B: contralateral TA muscle amplitudes.

Fig. 5. Mean percentage of trials with R1 responses pre- vs. post mucosal peel by animal. A: ipsilateral TA muscle responses. B: contralateral TA muscle responses.
magnitude as in the prepeel condition. It appears, therefore, that cricoarytenoid joint afferents do not contribute to the elicitation of the LAR. This conclusion is further strengthened in that servomotor displacement was readily observed to move the arytenoid cartilage and therefore would have activated cricoarytenoid joint receptors during both the pre- and postpeel conditions. Thus it is suggested that mucosal mechanoreceptors overlying the arytenoid complex represent the dominant source of somatosensory input needed to evoke the LAR in the cat.

Our investigation also found that the contribution of mucosal mechanoreceptors for eliciting the LAR was greatest in the posterior glottis. When the same displacement was administered to the mucosa overlying the thyroarytenoid muscle, no LAR responses occurred, and few occurred when stimulation was over the vocal process (Fig. 4). Because the magnitude of the reflex response grew with increased displacements of the arytenoid, the excitability of laryngeal motoneurons may be influenced by mechanical stimuli to this region. Collectively, these data are consistent with others' results. Immunohistochemical staining of intraepithelial nerve fibers in the epithelium of the laryngeal mucosa showed sharp territorial differences between the anterior and posterior regions of the glottis (20). These authors found that the posterior glottis contained the heaviest density of labeled fibers and suggested that this disproportionate distribution of fibers in the posterior glottis may be important for the perception of stimuli and the elicitation of reflexes in the larynx. Microneurographic recordings of ISLN afferent fibers in the cat by Davis and Nail (8) also showed greater density levels in the posterior glottis with a disproportionate number of RA mechanoreceptive afferents with small receptive fields over the arytenoid complex. Slowly adapting receptor profiles were also found but primarily populated the lumen of the larynx and to a lesser extent the aryepiglottic fold, the vocal process, and the base of the epiglottis. More importantly, Davis and Nail demonstrated that laryngeal mucosal receptors were acutely sensitive to both the static and dynamic features of indentation and were capable of faithfully after vibratory inputs up to 400 Hz. Given that the posterior glottal region normally undergoes rapid biomechanical adjustments for airway protection, a high distribution of afferents and their apparent high-frequency sensitivity in this region may provide for precise monitoring of sensory events during rapid laryngeal control. These data, in conjunction with our findings, suggest that mucosal afferents may effectively contribute to laryngeal sensorimotor responses. In addition, as the arytenoid cartilages are moved, the mechanoreceptors within the mucosa overlying them may operate as a primary source of sensory input necessary for laryngeal motor control.

An understanding of the distribution and integrity of ISLN-mediated afference in the human is of importance to normal and disordered laryngeal behavior such as chronic cough and irritable laryngeal disorders (22). Sensory abnormalities due to disease or injury of the human larynx have been suggested to form the basis for patient complaints such as foreign body sensations (22) and spasmodic dysphon (18). Abnormally heightened responses to typical laryngeal stimulation may be life threatening when laryngospasm causes airway obstruction and prolonged apnea (23–25, 39, 46). Unfortunately, few physiological studies address the contribution and central effects of general somatic afferents on laryngeal motoneuron pool excitability during coordinative actions in the human larynx. Behavioral data in humans suggest that mucosal afferents are sensitive to physiologically relevant inputs such as airflow, air pressure, and touch (2, 15, 36). Furthermore, Sanders and Mu (29) studied the territory supplied by the ISLN in excised human larynges and found a rich distribution of mechanoreceptive endings to the ventricular and vocal folds, the mucosa overlying the arytenoids, the posterior glottis, and the joint capsule of the cricoarytenoid joint. Similar distributions of ISLN afferent endings have been described for the cat larynx (54), suggesting that the cat may be an accurate model for testing the sensitivity of different receptor types contributing to movement dynamics during coordinated laryngeal behaviors.

Although our investigation has detailed the importance of mucosal afferents for evoking the LAR in the cat, this is not the only channel through which the LAR may be elicited. Stimulation of chemoreceptors, which are scattered throughout the laryngeal complex, will produce central apnea, alter respiratory rhythms, and elicit the LAR (6, 30, 47). Also, it is likely that other classes of receptors, such as joint or other mechanical receptors in muscle, were active during mucosal displacement. Although the LAR was not elicited after removal of the mucosa, these other receptors may have continued to be active. Without microneurography, however, the degree to which these afferents continued to be active after mucosal removal is not known.

The degree to which muscle spindles provide feedback to the motor neuron pool and alter muscle tone is unknown for the larynx. Few if any muscles spindles have been found in the intrinsic laryngeal muscles of primates and humans (3, 16, 19), and they seem to be absent in the cat. The extent to which muscle spindles are distributed and functionally active within the TA muscle of the human (3, 28) remains controversial. Because species may differ in the presence of muscle spindles and their possible physiological role in laryngeal control, caution must be taken in generalizing our results to the human. Regardless of these limitations, this study has provided data on the functional contribution and spatial sensitivity of mucosal mechanoreceptor afferents for the elicitation of the LAR in the cat. Continuing to differentiate the functional effects of each receptor class contained within the ISLN may help clarify the specific channels through which central changes may occur in humans with laryngeal disorders. This information is considered to have potential for understanding the pathophysiology of various sensorimotor laryngeal disorders.
Gratitude is expressed to Erich Luschei for technical assistance and advice, Frank Evans for the development of signal processing routines, and Carlos Cyrus for procedural support during data collection.

This project was funded by the Intramural Program at the National Institute of Neurological Disorders and Stroke, The National Institutes of Health, Bethesda, MD.

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