The Time-Course Closing Process of the Arytenoids and Laryngeal Vestibule with Fiberoptic Endoscopic Examination of Swallowing (FEES)

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ABSTRACT. In order to analyze the laryngeal movement during swallowing, we stimulated electrically the sensory branch of the superior laryngeal nerve (SLN) in rats and evaluated the time-course of movemment of the arytenoids and laryngeal vestibule with fiberoptic endoscopic examination of swallowing (FEES). The initiation time of laryngeal closure (ITLC) and the lasting time of laryngeal closure (LTLC) were compared between 24 normal rats and 20 unilateral SLN paralyzed rats. ITLC was the time between stimulation of the right sensory branch of the SLN and the beginning of the adductive movement of the arytenoids and laryngeal vestibule. LTLC was the time between the beginning of the adductive movement of the arytenoids and return of them. In this experiment, patterns of the adductive movement of the arytenoids and laryngeal vestibule occurred similarly in the unilateral SLN paralyzed group to the normal group. However, ITLC was delayed in rats with unilateral SLN paralysis compared to that in normal rats. There were no significant differences in LTLC between the paralyzed group and normal group. The present study suggests that ITLC is an important value for evaluating the function of protecting the lower respiratory tract during swallowing.

Key words: laryngeal movement — swallowing — dysphagia — superior laryngeal nerve (SLN) — fiberoptic endoscopic examination of swallowing (FEES)

Phylogenetically, the larynx developed as a sphincter to prevent tracheal penetration of the food during swallowing. Protection of the lower respiratory tract is accomplished by closure at the levels of the epiglottis, arytenoids, and vocal cords. However, details on the movement of the arytenoids and laryngeal vestibule during swallowing have not been reported. Although Ardran,[1] Logeman,[2] Saunders[3] and Ramsey[4] studied laryngeal closure during swallowing using videofluorography (VF), it was unclear whether they observed movements of individual structures of the larynx, such as the arytenoids and vocal cords because these movements are observed indirectly by VF. Therefore, I have clinically observed the time-course closing process of the arytenoids and laryngeal vestibule with fiberoptic endoscopic examination of swallowing (FEES), by which laryngeal closure can be macroscopically examined. In normal laryngeal movement, adduction and elevation of the arytenoids and laryngeal vestibule rapidly occur before laryngeal elevation. The vocal cords

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adduct simultaneously with laryngeal elevation, and the epiglottis turns over and closes the larynx.[5] Similar findings were reported by others, showing that closure of the arytenoids and laryngeal vestibule starts faster and continues for a longer time than closure of the vocal cords.[6,7]

For protection of the lower respiratory tract during swallowing, movement of the arytenoids and laryngeal vestibule is most important. There have been a few electrophysiological studies related to the movement of the arytenoids and laryngeal vestibule during laryngeal closure.[8] The time-course closing process of the arytenoids and laryngeal vestibule has not been measured precisely. Since there was no published experiments using rats analyzing the time-cource of laryngeal movement during swallowing, I stimulated the sensory branch of the superior laryngeal nerve (SLN) in rats and evaluated the movement of the arytenoids and laryngeal vestibule using FEES. The initiation time and lasting time of laryngeal closure in normal rats were compared to rats with unilateral SLN paralysis.

MATERIALS AND METHODS

As experimental animals, I used adult male Wistar rats among various mammals in which the larynx has a basic structure consisting of thyroid cartilage, epiglottal cartilage, annular cartilage, and cricoid cartilage. Of 64 rats, 8 rats died from bleeding while the SLN was detected. Other 12 rats were used to establish a method of stimulating the SLN of identifying the laryngeal structure. The remaining 44 rats (mean weight of 317 ± 30 g, 280-370 g) were used in the experiment. The rats were divided into a normal group consisting of 24 (mean weight of 320 ± 27 g) and a unilaterally SLN-paralyzed group consisting of 20 (mean weight of 310 ± 30 g). The following experiment was approved by the Kawasaki Medical School Animal Study Committee (No. 00-077) and performed in accordance with the Guidance for Animal Studies of Kawasaki Medical School.

In the experimental procedure, rats were anesthetized with an intraperitoneal injection of 0.5 ml/kg sodium pentobarbital (Somnopentyl®), and fixed in the supine position. The neck was shaved and a midline incision was made to expose the trachea from the hyoid to the sternal notch. After tracheotomy, respiration was managed at a ratio of nitrous oxide 1 to oxygen 0.5 using an artificial ventilator (SAR-830 Ventilator; Dwyer®). The sternocleidmastoid muscles were retracted laterally and the recurrent laryngeal nerves (inferior laryngeal nerves) were identified several millimeters below the cricoid cartilage.

The sensory and motor branches of the SLN were identified just superior and lateral to the cricoid cartilage. To induce a swallowing movement electrically, the right sensory branch of the superior laryngeal nerve was stimulated using a fishhook-shaped bipolar electrode. The duration of the electrical stimulation was 0.2 ms, and the stimulus intensity was 5 mA. The nerve was stimulated 10 times for 10 minutes (Fig 1). Simultaneously, the arytenoids and laryngeal vestibule were directly visualized by a fiberoptic endoscopy (OLYMPUS T61-4994) inserted through the oral cavity. All of the

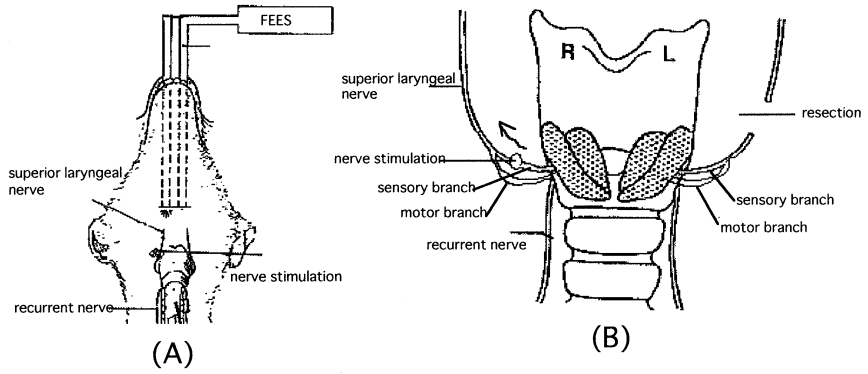

Fig 1. Schematic representation of experimental procedure. (A) experimental set-up. (B) section of the common branch of the left superior laryngeal nerve. An arrow (←) means the direction of conduction of electrical current.

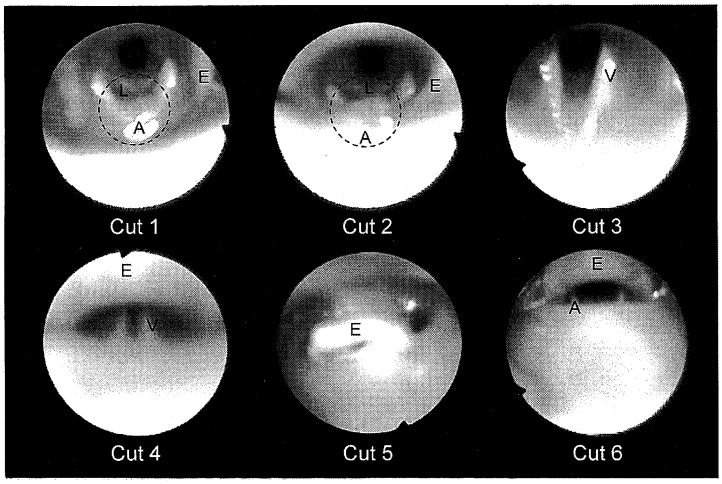

Fig 2. Images of the laryngeal cavity in response to stimulation of the sensory branch of the SLN in the normal rat. That movement process of laryngeal closure shows from Cut 1 to Cut 3, and the adductive movement of vocal cords shows from Cut 3 to Cut 4.

Before the adductive movement of the arytenoids, the aryepiglottic folds showed U-shape (Cut 1). The shape of the aryepiglottic folds changed to box-shape during their movement (Cut 2).

A : arytenoids, L : laryngeal vestibule, E : epiglottis

images were recorded using a digital video (Fig 2). The signal of the electrical current was converted to a sound to record the stimulating point simultaneously on the videotape. The paralyzed group was prepared by cutting the left common branch of the SLN (Fig 1B). The above-mentioned experiments were performed in the normal control group and the paralyzed group.

The time was measured from the electrical stimulation to beginning of the adductive movement of the arytenoids. This period was defined as initiation time of laryngeal closure (ITLC). Moreover, the time between beginning of the

adductive movement of the arytenoids and return of them was measured. This time was defined as the lasting time of laryngeal closure (LTLC). Each frame of the digital video record was analyzed. The beginning and movement process of the arytenoids and the laryngeal vestibule in response to electrical in a normal rat is shown in Fig 2. Before the adductive movement of the arytenoids, the aryepiglottic folds showed U-shaped. During their movement, the shape of the aryepiglottic folds changed to box-shape. The beginning of the change was judged by sending each frame backward and forward. ITLC and LTLC in each rat were calculated by averaging 10 records of the values. Standard error (SE) of these values in each rat was calculated as the percentage. Mean ITLC and mean LTLC were compared between the normal group and the paralyzed group. For statistical analysis, the Welch's t-test was used, and a p-value <0.05 was defined as significant.

RESULTS

Laryngeal closure was first at the adductive movement of the arytenoids and laryngeal vestibule, next at the adductive movement of the vocal cords in the normal group (Fig 2). Downward bending movement of the epiglottis was not found clearly in rats. This downward bending movement of the epiglottis was quite different from the human. The pattern of the laryngeal closure was similar to the paralyzed group. The adductive movement of the arytenoids and laryngeal vestibule preceded the adductive movement of the vocal cords also in this group.

ITLC was 193 ± 9 msec and mean SE was $9.5\pm2.4\%$ in the normal group. ITLC was 274 ± 113 msec and mean SE was $18.9\pm4.5\%$ in the paralyzed group. ITLC in the paralyzed group was significantly longer than that in the normal group (Fig 3). LTLC was 907 ± 76 msec and mean SE was $10.4\pm6.9\%$ in the normal group. LTLC was 967 ± 116 msec and mean SE was $14.5\pm4.2\%$ in the paralyzed group (Fig 4). There was no significant difference in LTLC between the two groupes.

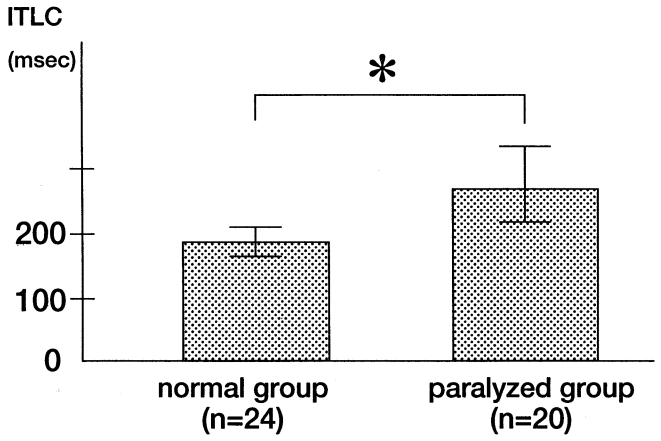

Fig 3. Comparision of the initiation time of laryngeal closure (ITLC) between normal group and unilateral SLN paralyzed group.
Single asterisk (*) shows $P<0.05$.

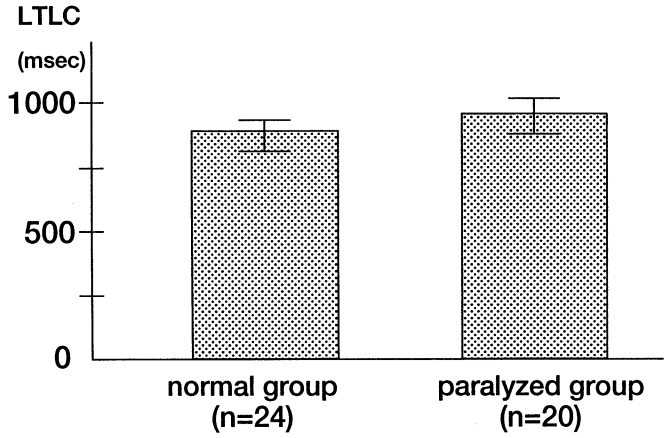

Fig 4. Comparision of the lasting time of laryngeal closure (LTLC) between normal group and unilateral SLN paralyzed group.

DISCUSSION

The results of FEES in stroke patients with impaired laryngeal closure correlated highly with aspiration detected by VF.[5] Laryngeal closure of the arytenoids and laryngeal vestibule was known to begin before adductive movement of the vocal cords and closure of the epiglottis. Moreover, it continues longer than closure by the epiglottis. In the present study related to the laryngeal closure, the adductive movement of the arytenoids and laryngeal vestibule was shown to occur before adductive movement of the vocal cords also in the rats. However, the epiglottis scarcely moved. During the adductive movement of arytenoids and laryngeal vestibule, the transverse arytenoids muscles and oblique arytenoids muscles innervated by the motor branch of the SLN were said to contract simultaneously. These muscles pull the bilateral arytenoid cartilages to the center of the laryngeal cavity. Then, the vocal cords are adducted by the contraction of the bilateral cricoarytenoid muscles innervated by the recurrent laryngeal nerve. FEES was supposed to be useful examination for observing the process of the adductive movement of the arytenoids and laryngeal vestibule.

In this experiment, the laryngeal movement was evoked by the stimulation of the sensory branch of the SLN. The SLN branches off from the vagus and the sensory branch of the SLN act as an important afferent for swallowing. Sensory distribution of this branch spreads over the larynx and epiglottis.[9] There are a lot of sensory receptors of this nerve especially in the arytenoids region.[10] The impulses of the electrical stimulation to the sensory branch of the SLN are transmitted to the reticular formation via the nucleus solitarius in the medulla oblongata. The efferent impulses are transferred to the nucleus ambiguous and other motor nucleuses. The nucleus ambiguous activates the motor fibers of the glossopharyngeal and vagal nerves.[11] The precise nuclear connections in the brain stem were not made clear. In the present study, the common branch of the SLN was cut off as a substitute for a model of medullary infarction. I compared laryngeal movement in the rats with paralysis of the unilateral common branch of the SLN to that in the normal rats.

Although the process of the adductive movement of the arytenoids and laryngeal vestibule was the same as that in the normal rat group, ITLC was delayed in the unilateral SLN paralyzed group. As for humans, Karin *et al*[8] reported that the speed of laryngeal movement was significantly slower in patients with unilateral SLN paralysis than normal controls. Trapp *et al* also compared SLN paralyzed dogs and normal dogs, and showed the delay of the initiation of laryngeal closure.[12] These reports were consistent with our results. The reason of the delayed ITLC may be the paralysis of the unilateral arytenoids region. Since the sound side of the arytenoids moved powerfully, pattern of the laryngeal movement might be observed as if it was normal. The arytenoids of the rats were too small to observe the fine movement with FEES. The arytenoids and vestibule of larynx adduct and move upward rapidly is quite different from the human study.[5] In this animal experiment, LTLC in the paralyzed group was not different significantly from the normal group. It may be due to the powerful movement of the sound arytenoids. In addition, the abduction and depression of the arytenoids are performed passively without activation of the SLN. In future study, the recordings of the electromyography from the laryngeal muscles related to swallowing should be done for clarifying the mechanism of laryngeal movement. Although the swallowing reflex was said to be affected by the depth of general anesthesia, the stimulation of the SLN could evoke the swallowing reflex including both laryngeal closure and adductive movement of vocal cords.[13] Since there have been some opinions that decerebration should be done for induction of the swallowing reflex, studies are further necessary to make clear the influence of decerebration. Moreover, a direct model of medullary infarction should be made in future studies.

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