Pharyngoglottal closure reflex: identification and characterization in a feline model

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Medical College of Wisconsin Dysphagia Institute and the Division of Gastroenterology and Hepatology, Departments of Medicine, Neurology, and Otolaryngology, Medical College of Wisconsin and Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin 53226

Shaker, Reza, Bidyut K. Medda, J unlong Ren, Safwan Jaradeh, Pengyan Xie, and Ivan M. Lang. Pharyngoglottal closure reflex: identification and characterization in a feline model. Am. J. Physiol. Gastrointest. Liver Physiol. 275: G521–G525, 1998.—Earlier studies in humans have shown that pharyngeal stimulation by water at a threshold volume induces a brief vocal cord adduction, i.e., pharyngoglottal closure reflex. The present study was undertaken to 1) develop a suitable animal model for physiological studies of this reflex and 2) delineate its neural pathway and effector organs. Studies were done in cats by concurrent videendoscopy and manometry followed by electromyographic studies. At a threshold volume (0.3 ± 0.06 ml), injection of water into the pharynx resulted in a brief closure of the vocal folds, dosing the introitus to the trachea. Duration of this closure averaged 1.1 ± 0.1 s. Bilateral transection of the glossopharyngeal nerve completely abolished this reflex but not swallows induced by pharyngeal water stimulation. The pharyngoglottal closure reflex is present in the cats. The glossopharyngeal nerve is the afferent pathway of this reflex, and the interarytenoid and lateral cricoarytenoid muscles are among its target organs.

Methods

Previous studies in humans (3) have shown that stimulation of pharyngeal mucosa by injection of minute amounts of water at a threshold volume induces a brief vocal cord adduction, indicating the existence of a pharyngoglottal closure reflex. Sudden entry of liquid stimulates complete vocal cord closure, whereas gradual entry induces partial vocal cord closure. It is possible that this reflex may play a contributory role in airway protection against aspiration during premature spill of swallowed material, as well as retrograde entry of gastric refluxate into the pharynx. The neural pathway that mediates this reflex, however, has not been determined. In addition, an animal model is needed for physiological studies of the pharyngoglottal closure reflex and 2) identify the neural pathway and effector organs of this reflex.

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Methods

Studies were approved by the Animal Care Committee of the Medical College of Wisconsin and were done in two stages. Stage 1: Concurrent videendoscopic and manometric studies. During this stage, we studied 10 cats of either sex, weighing 2–4 kg, by concurrent videendoscopic and manometric technique. The animals were anesthetized using intramuscular injection of 4 mg/kg Telazol (tiletamine HCl and zolazepam HCl; Avelco, Fort Dodge, IA). To record the glottal function an endoscope (GIF-XV10 Olympus, Lake Success, NY) of 11-mm diameter and 100° angle of view was passed transorally through a bite block and positioned at the level of the free margin of the epiglottis. With the optical head of the scope in this position, the vocal folds, arytenoids, posterior surface of the epiglottis, laryngeal vestibule, pharyngeal wall, and the area of the upper esophageal sphincter (UES) opening were visualized. Endoscopic images were recorded on 0.5-in. videotape using a super VHS video recorder (AG1960; Panasonic, Secaucus, NJ) that recorded at 30 frames/s.

To monitor the UES pressure, we used a manometric assembly that incorporated a sleeve device (60 × 6 × 4 mm). The assembly had recording side holes at the proximal and distal margins of the sleeve for manometric positioning. The sleeve and manometric channels were infused with distilled water (0.3 ml/min) using a minimally compliant pneumohydraulic system (Arndorfer Specialties, Greendale, WI), and pressure tracings were recorded on an eight-channel polygraph recorder (Grass Instruments, Quincy, MA). To prevent pharyngeal and glottal stimulation, the pharyngeal port was not infused after the sleeve device was positioned within the UES.

To stimulate the pharyngeal receptors, we injected incrementally (0.1 ml) increasing volumes of water colored with food dye, directed posteriorly into the pharynx. Injections were done via a designated port on the manometric assembly 1.5 cm above the sleeve. The interval between injections ranged between 4 and 8 min. We started with injections of 0.1 ml of water and increased the injection volume until a swallow occurred. The injection port was connected to the chart recorder via an extracorporeal transducer, thus each injection induced a pen deflection that was used to correlate the glottal response recorded on videotape with pharyngeal water injection. Manometric and video endoscopic recordings were synchronized using a specially designed timer (Thalner Electronics, Ann Arbor, MI), and each volume was tested three times.

Stage 2: EMG studies. Experiments in this stage were carried out on seven cats of either sex weighing 2.0–4.0 kg. Under ketamine anesthesia (20 mg/kg; ketamine HCl, Aveco), animals were prepared with venous and arterial lines, and carotid arteries were ligated bilaterally. The trachea was severed 2 cm below the cricoid cartilage, and animals were intubated and given 0.6–1.0% halothane anesthesia for midcollicular decerebration. After decerebration, halothane was discontinued and the animals breathed room air spontaneously and did not receive any anesthetics. Temperature was maintained at 37–38°C by an external heating pad. Mean arterial blood pressure was kept >80 mmHg by intravenous infusion of 5% dextrose in 0.9% NaCl solution, as required. Laryngeal adductor and cricopharyngeus (CP) muscles were exposed through midline incision. The electromyographic (EMG) activity of the right lateral cricoarytenoid and the muscle equivalent to human interarytenoid muscles were
Fig. 1. Still frame from videendoscopic recording of vocal fold closure response to pharyngeal water stimulation. A: cat glottis immediately before the injection of 0.3 ml of colored water into the pharynx directed posteriorly. Vocal folds are fully open and free of color staining. B: vocal folds are completely adducted 0.1 s after the injection of 0.3 ml of colored water into the pharynx. The glottis remains free of blue staining, indicating that the injected water has not come in contact with the glottis. VF, vocal folds; T, introitus to trachea; P, pyriform sinus; PH, posterior pharyngeal wall; M, manometric catheter.
monitored using stainless steel wires (Grass E-2 or Medwire AS632) embedded in the muscles. We recorded bipolar (inter-electrode distance of 3–5 mm) EMG activity from the interarytenoid (human equivalent) muscle, but because of the small size of the lateral cricoarytenoid muscles we recorded EMG activity from a single electrode in the muscle and an indifferent electrode on the skin. The CP EMG activities were recorded using bipolar electrodes (AS632). The EMG signals were high-pass filtered at 100 Hz and amplified 10 times using an AC preamplifier. The EMG signal was fed into a Grass model 7P3 preamplifier and integrator, where the signal was further amplified, full-wave rectified, and electronically integrated at a time constant of 0.25. The output was calibrated using the internal 500-Hz test signal, and the threshold level was set just below the spontaneous EMG level at the end of expiration. The 0.5-A high filter frequency was set at 0.5 kHz and 3 Hz for the raw EMG signal and the integrated EMG signal, respectively. Integrated and raw EMG signals were recorded simultaneously on a Grass multichannel polygraph machine. The raw and integrated EMG responses were also digitized and stored on the hard drive of a computer (IBM clone, 120 MHz Pentium processor) using a computerscope (RC Electronics, Goleta, CA), hardware, and software. Experimentation was started at least 2 h after the cats were decerebrated and pharyngeal water stimulation (described above) was repeated, and EMG activities of the glottal adductor muscles were recorded.

The occurrence of swallow was determined by a signal recorded through the nonperfused pharyngeal port, as well as the characteristic loss of CP tone seen on CP-EMG recordings.

After obtaining control responses to pharyngeal water stimulation, the glossopharyngeal nerves were transected bilaterally. Ten minutes after nerve transection the reflex responses were tested again, and changes in responses were recorded.

RESULTS

Videoendoscopic studies. Real-time and frame-by-frame analysis of the videoendoscopic recordings showed that at a threshold volume (0.3 ± 0.1 ml) injection of water into the pharynx directed posteriorly resulted in a brief closure of the vocal folds, completely closing the laryngeal vestibule. During swallowing, due to vestibular closure and pharyngeal contraction, the larynx was closed and vocal folds were obscured; they remained visible during initial and final stages of swallow-induced vocal fold kinetics. The pharyngoglottal closure reflex, the laryngeal vestibule remained open, thus vocal fold kinetics were visible during the entire event. Both total duration (1.1 ± 0.1 s) and duration of complete closure (0.87 ± 0.12 s) for pharyngoglottal closure reflex were significantly shorter than those for pharyngeal swallows (1.8 ± 0.3 s and 1.47 ± 0.24 s, respectively) (P < 0.05).

![Fig. 2. Vocal fold kinetics during pharyngoglottal closure reflex (PGCR; A) and pharyngeal swallow (PSW; B). VC-Ad-O, onset of closure of vocal folds; VC-Ad-Max, maximum closure of vocal folds; VC-Ab-O, onset of opening of vocal folds; VC-Ab-Max, return of vocal folds to resting open position. During swallowing, due to vestibular closure and pharyngeal contraction, laryngeal vestibule was closed and vocal folds were obscured; they remained visible during initial and final stages of swallow-induced vocal fold kinetics. During the pharyngoglottal closure reflex, the laryngeal vestibule remained open, thus vocal fold kinetics were visible during the entire event. Both total duration (1.1 ± 0.1 s) and duration of complete closure (0.87 ± 0.12 s) for pharyngoglottal closure reflex were significantly shorter than those for pharyngeal swallows (1.8 ± 0.3 s and 1.47 ± 0.24 s, respectively) (P < 0.05).](http://ajpgi.physiology.org/)

![Fig. 3. Example of the effect of pharyngeal water stimulation on myoelectrical activity of the interarytenoid (human equivalent), lateral cricoarytenoid, and cricopharyngeus muscles (CP). Whereas pharyngeal water injection resulted in contraction of the glottal adductor as well as CP muscles (A and B), swallows triggered by pharyngeal water injection resulted in contraction of glottal adductors and relaxation of the CP muscles (C and D). This relaxation, however, was followed by a postdeglutitive contraction. IA, interaryt enoid muscle; LCA, lateral cricoarytenoid muscle; EMG, raw electromyographic activity; ò, integrated EMG activity.)](http://ajpgi.physiology.org/)
introitus to the trachea (Fig. 1). The vocal fold closure was not accompanied by movement of the closed glottis toward the epiglottis. Analysis of videendoscopic recordings also demonstrated the exit of colored water from the injection port staining the posterior pharyngeal wall without contacting the glottis.

Further increase in the volume of injected water resulted in swallowing. The average volume of water that induced swallowing (1.0 ± 0.3 ml) was significantly larger than that of the threshold for stimulation of the pharyngoglottal closure reflex. In each animal, injection of volumes larger than the threshold volume for activation of the reflex, but smaller than that of swallowing, consistently activated the pharyngoglottal closure reflex.

Duration of glottal closure from the onset of closure to its return to resting position, induced by injection of the threshold volume, averaged 1.1 ± 0.1 s. This duration did not increase with injection of volumes larger than the threshold volume. Duration of glottal closure induced by swallowing (1.8 ± 0.3 s) was significantly longer than that of the pharyngoglottal closure reflex (P < 0.05). This difference was due to a longer duration of the complete adduction period for pharyngeal swallow compared with the pharyngoglottal closure reflex (Fig. 2). The time interval between the onset of closure to maximum closure and onset of opening to full opening of the vocal folds for both functions was similar.

EMG studies. Pharyngeal water injected at the threshold volume, determined in endoscopic studies, induced myoelectrical activities in both studied glottal adductor muscles (Fig. 3, A and B). In addition, it frequently stimulated the CP muscle. On the other hand, whereas pharyngeal swallow resulted in contraction of glottal adductor muscles, it induced a brief relaxation of the CP, followed by its contraction (Fig. 3, C and D).

For both glottal adductor muscles studied, EMG activities induced by the pharyngoglottal closure reflex were significantly less compared with that of swallowing (P < 0.05; see Table 1). Bilateral transection of the glossopharyngeal nerves completely abolished the pharyngoglottal closure reflex but not swallows induced by pharyngeal water stimulation (Fig. 4).

**DISCUSSION**

In this study we identified and characterized the existence of a pharyngoglottal closure reflex in cats. A similar reflex has been previously identified in humans (3). Previous investigation of another airway closure reflex, esophagoglottal closure reflex (5), has shown interspecies variability in regard to its existence. These studies documented this reflex in humans, cats, and monkeys but not in opossum (6). In the current study other species were not investigated and the interspecies variation of the pharyngoglottal closure reflex remains to be investigated.

The afferent arm of this reflex seems to include the glossopharyngeal nerve, because bilateral transection of this nerve abolishes the reflex. The central control mechanism, although undoubtedly located in the brain stem, has not been completely studied. Because glottal closure is known to be mediated through the recurrent laryngeal nerve, the efferent limb of the pharyngoglottal closure reflex undoubtedly includes this nerve. The effector organs include the glottal adductor muscles such as interarytenoid (human equivalent) and lateral cricoarytenoid muscles. The possible participation of other adductor/tensor muscles of the larynx, such as the vocalis, thyroarytenoid, and cricothyroid muscles awaits investigation.

It is known that tactile stimulation of the glottis induces its closure (9). In this study, using colored water, it was ascertained that the injected water did

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<th>Table 1. EMG activities of glottal adductor muscles</th>
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<td>Integrated IA-EMG, mV/Hz</td>
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Values are means ± SE; n = 7. IA, interarytenoid muscle; EMG, electromyographic; LCA, lateral cricoarytenoid muscle. *P < 0.05.

Fig. 4. Example of effect of bilateral transection of the glossopharyngeal nerves on pharyngoglottal closure reflex and pharyngeal swallow. Intrapharyngeal injection of any volume of water did not elicit any myoelectrical activity in the interarytenoid or lateral cricoarytenoid muscles following bilateral transection of the glossopharyngeal nerves. On the contrary, stimulation of pharyngeal swallow remained intact after glossopharyngeal transection and resulted in contraction of the above-mentioned glottal adductor muscles and relaxation followed by contraction of the CP muscle. Abbreviations defined in legend for Fig. 3.
not come in contact with the glottis, thus assuring that the posterior pharyngeal wall was stimulated and not the glottis. Previous studies have documented various pharyngeal receptive fields capable of stimulating pharyngeal reflexive swallow. These fields include posterior tonsillar pillars, epiglottis, larynx, and posterior pharyngeal wall (2, 7, 8). In the present study contact of larger volumes of colored water than threshold volume for the pharyngoglottal reflex also initiated swallowing, indicating that various reflexes may be evoked from similar receptive fields in the pharynx. Whether similar or different groups of receptors mediate the various reflexes arising from a single receptive field is not currently known.

It is known that glottal closure is mediated through recurrent laryngeal nerves (4). The findings of this study indicate that the pharyngoglottal closure reflex and the glottal closure during pharyngeal (reflexive) swallow share similar afferent nerves (recurrent laryngeal nerve) as well as target organs. Our finding that bilateral transection of the glossopharyngeal nerve abolished this reflex, but reflexive swallowing and its associated glottal closure still remained intact suggests that 1) contrary to swallowing that may be initiated by multiple and complex afferent pathways including the glossopharyngeal and superior laryngeal nerves, the pharyngoglottal reflex seems to be initiated through a single afferent pathway via the glossopharyngeal nerve and 2) although the pharyngoglottal closure reflex shares its afferent and efferent pathways with the swallowing reflex, it probably has its reflex circuitry functionally distinct from that of swallowing.

Our finding that a larger volume of injected water was required to trigger a reflexive swallow compared with that of the pharyngoglottal closure reflex suggests either the involvement of different types of receptors for mediating these two reflexes or activation of a larger number of the same receptor types for triggering swallowing than for triggering the pharyngoglottal closure reflex.

Previous studies in humans (3) have documented a smaller threshold volume for triggering the pharyngoglottal closure reflex compared with the threshold volume determined in this study in cats. This difference, in addition to interspecies variation, could potentially be due to the effect of anesthetics used in our current animal studies. However, in both species the duration of closure does not increase by increasing the volume of injected water, indicating a stereotypical “all or none” response.

The physiological role of the pharyngoglottal closure reflex in cats has not been studied completely; however, previous reports in humans indicate that it is triggered when a portion of the oral bolus is spilled into the pharynx and contacts the pharyngeal wall during the preparatory phase of swallowing (1). In addition, it is postulated that in humans this reflex may be triggered during reflux of gastric content into the pharynx, thereby preventing aspiration by closing the introitus to the trachea.

In summary, the pharyngoglottal closure reflex is present in the feline species. The threshold volume for triggering this reflex and the EMG activities of its target organs are significantly lower than that of a reflexive (pharyngeal) swallow. The glossopharyngeal nerve is the afferent pathway of this reflex and the interarytenoid (human equivalent) and lateral cricoarytenoid muscles are among its target organs.

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REFERENCES