Electromyographic activity of the hyoepiglotticus muscle and control of epiglottis position in horses

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**Objective**—To determine whether the hyoepiglotticus muscle has respiratory-related electromyographic activity and whether electrical stimulation of this muscle changes the position and conformation of the epiglottis, thereby altering dimensions of the aditus laryngis.

**Animals**—6 Standardbred horses.

**Procedure**—Horses were anesthetized, and a bipolar fine-wire electrode was placed in the hyoepiglotticus muscle of each horse. Endoscopic images of the nasopharynx and larynx were recorded during electrical stimulation of the hyoepiglotticus muscle in standing, unsedated horses. Dorsoventral length and area of the aditus laryngis were measured on images obtained before and during electrical stimulation. Electromyographic activity of the hyoepiglotticus muscle and nasopharyngeal pressures were measured while horses exercised on a treadmill at 50, 75, 90, and 100% of the speed that produced maximum heart rate.

**Results**—Electrical stimulation of the hyoepiglotticus muscle changed the shape of the epiglottis, displaced it ventrally, and significantly increased the dorsoventral length and area of the aditus laryngis. The hyoepiglotticus muscle had inspiratory activity that increased significantly with treadmill speed as a result of an increase in phasic and tonic activity. Expiratory activity of the hyoepiglotticus muscle did not change with treadmill speed in 4 of 6 horses.

**Conclusions and Clinical Relevance**—Findings reported here suggest that contraction of the hyoepiglotticus muscle increases dimensions of the airway in horses by depressing the epiglottis ventrally, and significantly increased the dorsoventral length and area of the aditus laryngis. The hyoepiglotticus muscle increases dimensions of the aditus laryngis by depressing the epiglottis ventrally, and laterally by the aryepiglottic folds. The position of the epiglottis is controlled by the position of the larynx and hyoid apparatus, especially during swallowing. The hyoid and lingual muscles pull the larynx rostroventrally during swallowing so that the laryngeal opening is under the base of the tongue. This motion and the movement of the tongue push the epiglottis against the laryngeal entrance as the bolus of food moves caudally over the larynx through the esophagus. At the conclusion of swallowing, the larynx moves caudally and the tongue relaxes, and the epiglottis returns to a normal position either passively or via contraction of the hyoepiglotticus muscle. The hyoepiglotticus muscle is the only muscle that attaches to the epiglottis. The hyoepiglotticus muscle involves the extrinsic laryngeal muscle that originates on the basihyoid bone in horses and on the ceratohyoid bones in cattle and dogs, and it inserts on the ventral body of the epiglottis. On the basis of our observations during dissection of the laryngeal area of the cadavers of several adult horses, the hyoepiglotticus muscle inserts on the ventral surface of the body of the epiglottis by an attachment approximately 12 to 15 mm in length on the long axis of the epiglottis and 8 to 10 mm in width. The insertion and body of the hyoepiglotticus muscle can be palpated during oral examination of anesthetized horses.

In dogs, the hyoepiglotticus muscle has inspiratory- and expiratory-related electromyographic activity that is augmented during increased respiratory drive. Vigorous recruitment of the hyoepiglotticus muscle disrupts the seal between the soft palate and epiglottis in dogs, displacing the epiglottis ventrally against the tongue to dilate the oral pathway for airflow. The change from nasal to oral breathing allows dogs to pant, which is important in cooling and decreasing respiratory resistance to airflow. We hypothesized that contraction of the hyoepiglotticus muscle in horses is its epithelium, and also may be important in determining nasal-oral airway partitioning. In many mammals, excluding humans, the epiglottis and soft palate overlap such that the ventral surface of the epiglottis contacts the dorsal surface of the soft palate, effectively separating the nasopharyngeal and oral airways. The epiglottis may help to form a tight seal that locks the larynx into the nasopharynx to preserve nasal breathing. The functional advantage of this separation may be the preservation of olfactory processes to detect predators during feeding, an important defense mechanism in horses.

The epiglottis forms part of the vestibule or entrance to the larynx (ie, aditus laryngis). The aditus laryngis is bound rostrally by the epiglottis, caudally by the arytenoid cartilages, and laterally by the aryepiglottic folds. The position of the epiglottis is controlled by the position of the larynx and hyoid apparatus, especially during swallowing. The hyoid and lingual muscles pull the larynx rostroventrally during swallowing so that the laryngeal opening is under the base of the tongue. This motion and the movement of the tongue push the epiglottis against the laryngeal entrance as the bolus of food moves caudally over the larynx through the esophagus. At the conclusion of swallowing, the larynx moves caudally and the tongue relaxes, and the epiglottis returns to a normal position either passively or via contraction of the hyoepiglotticus muscle. The hyoepiglotticus muscle is the only muscle that attaches to the epiglottis. The hyoepiglotticus muscle involves the extrinsic laryngeal muscle that originates on the basihyoid bone in horses and on the ceratohyoid bones in cattle and dogs, and it inserts on the ventral body of the epiglottis. On the basis of our observations during dissection of the laryngeal area of the cadavers of several adult horses, the hyoepiglotticus muscle inserts on the ventral surface of the body of the epiglottis by an attachment approximately 12 to 15 mm in length on the long axis of the epiglottis and 8 to 10 mm in width. The insertion and body of the hyoepiglotticus muscle can be palpated during oral examination of anesthetized horses.

Despite its prominent location at the entrance to the laryngeal airway, little is known about the function of the epiglottis. It has been regarded as a protective structure for the airway during swallowing to prevent food from entering the trachea, although this may be inaccurate because many species that swallow lack an epiglottis. The epiglottis may be an accessory olfactory organ, based on the fact it has olfactory receptors with
pulls the epiglottis toward the basal hyoid bone and depresses it against the soft palate. This action likely enlarges the aditus laryngis, potentially resulting in decreased respiratory resistance to airflow, which is important during strenuous breathing efforts in obligate nasal breathers such as horses. Therefore, the hypothesis of the study reported here was that the hypoepiglotticus muscle is an important muscle for dilating the respiratory passages in horses. It functions to increase the aditus laryngis by depressing the epiglottis ventrally against the soft palate, which may change the conformation of the epiglottis.

### Materials and Methods

**Horses**—Six Standardbred horses (2 geldings and 4 mares) that were 3 to 8 years old and weighed between 423 and 588 kg were used in the study. Results of physical examinations of the horses during rest and endoscopic examinations of the larynx and nasopharynx of horses during rest and exercise on a high-speed treadmill were unremarkable. The study was approved by the All-University Committee on Animal Use and Care at Michigan State University.

**Exercise protocol**—All horses were trained to run on a treadmill prior to initiation of the experiment. Prior to the study, maximum heart rate (HRmax) was determined during an incremental exercise test that consisted of a warm-up period of 3 minutes at 4 m/s, 2 minutes at 6 m/s, and 1 minute at each of 8, 10, 11, 12, and 13 m/s or until the horse became fatigued and could not maintain its position on the treadmill despite humane encouragement. During the test, heart rate was recorded by use of a telemetry system despite humane encouragement. During the test, heart rate was recorded by use of a telemetry system during the last 15 seconds at each speed. Heart rate was plotted against treadmill speed, and speeds corresponding to 50% of HRmax (HRmax50), 75% of HRmax (HRmax75), and 90% of HRmax (HRmax90) were determined from the curve.

**Electrode placement**—Horses were medicated by administration of xylazine hydrochloride (0.04 mg/kg, IV), and anesthesia was induced by administration of ketamine hydrochloride (2.2 mg/kg, IV) and diazepam (0.1 mg/kg, IV). Endotracheal intubation was performed, and anesthesia was maintained by administration of isoflurane in oxygen. Horses were positioned in dorsal recumbency, and the ventral area of the throat was prepared for aseptic surgery. An incision was made beginning 2 cm rostral to the basihyoid bone, and it extended caudally for 8 cm. Blunt dissection through the pharyngeal fat exposed the hypoepiglotticus ligament and muscles as they extended from the basihyoid bone to the ventral surface of the epiglottis. Bipolar fine-wire electrodes and a ground wire were constructed from fluoride-coated wire. A small amount of resin cement was applied to the end of each electrode so it could be seated in a muscle. The electrode was seated in the hypoepiglotticus muscle, and correct positioning of the electrode was confirmed by observing contraction of the muscle during stimulation with a unilateral tetanic low-voltage stimulus (4 to 6 V; duration of 2 seconds; 40 pulses/s; 0.2 ms/pulse). During muscle stimulation, movements of the epiglottis were observed through an endoscope. Ventral displacement of the epiglottis during electrical stimulation confirmed correct positioning of the electrode. A ground wire was placed in the subcutaneous space just over the left sternocephalicus muscle.

**Experimental protocol**—The day after electrode placement, electrical stimulation of the hypoepiglotticus muscle was performed in standing, unsedated horses. An endoscope was passed through the right nostril into the nasopharynx such that the epiglottis and aditus laryngis could be seen clearly. The hypoepiglotticus muscle was stimulated (40 pulses/s; pulse duration of 0.2 milliseconds; train duration of 5 seconds). Initial stimulus voltage was 2 V, and it was incrementally increased to 10 to 13 V or until the horse began to swallow. All endoscopic examinations were recorded on videotape.

Following electrical stimulation, a 150-cm polyethylene side-hole catheter (polyethylene tubing; inside diameter of 2.15 mm; outside diameter of 3.25 mm) was placed through the right nares and secured to the muzzle of the horse with tape. The catheter was constructed with 6 holes on the side of the tubing beginning a distance that was 8 times the catheter diameter from the sealed tip. The catheter tip was placed in the region of the nasopharyngeal opening of the eustachian tube in the nasopharynx and was connected to a differential pressure transducer that was calibrated (calibration range, 5 to 25 cm H2O) before each experiment by use of a water manometer. Each horse then completed an incremental exercise test that consisted of a warm-up period of 3 minutes at 4 m/s followed by periods of 1 minute each at speeds that corresponded to HRmax50, HRmax75, HRmax90, and HRmax100. Nasopharyngeal pressure and electromyography (EMG) signals were recorded throughout the experiment on a computer and a physiologic recorder. The electrode was removed from the hypoepiglotticus muscle of each horse following completion of the exercise test.

**EMG measurements**—The EMG signals were processed through a sixth-order Butterworth filter (band pass, 50 to 5,000 Hz), amplified, rectified, and time-averaged with a constant of 100 milliseconds. Both raw EMG and moving time-averaged signals were recorded. Quantification of the EMG was performed by digitization of the moving time-averaged EMG signal. Mean electrical activity of each moving time-averaged waveform was determined by dividing the total area of each waveform by duration of the electrical activity. This method of signal quantification permitted comparison of muscle activity during conditions in which inspiratory or expiratory times changed as respiratory frequency increased with increases in treadmill speed. To standardize EMG activity among horses or among experimental days, all activity was expressed as a ratio of the muscle’s activity during HRmax50. Timing of EMG activity of the hypoepiglotticus muscle was related to nasopharyngeal pressure and was measured by use of the raw EMG signal. The EMG lead-time for the hypoepiglotticus muscle was measured as the interval from the onset of phasic inspiratory activity to the onset of a decrease in nasopharyngeal pressure.

**Aditus laryngis measurements**—Videotapes of endoscopic examinations recorded during breathing while at rest and during stimulation of the hypoepiglotticus muscle were reviewed, and images were printed. To help ensure consistency in measurement within each horse, multiple images were selected from portions of the videotape where the endoscope had not moved within the nasopharynx and the corniculate processes of the arytenoids were in a similar degree of abduction. Images that were to be analyzed were scanned into a computer by use of a graphics software program. Length of the right corniculate process of the arytenoid cartilage was measured and compared between the stimulated and unstimulated image for each horse, and pairs of images were selected for measurement when length of the right arytenoid cartilage was the same for the stimulated and unstimulated images. This was done in an attempt to standardize the size of the larynx between the 2 images. Once pairs of stimulated and unstimulated images were selected for each horse, the dorsoventral length of the aditus laryngis was obtained by measuring the distance on a perpendicular line...
from the dorsal aspect of the aditus laryngis (at the point where the right and left corniculate processes of the arytenoid cartilages met) to the epiglottis. For each horse, length of the aditus laryngis during electrical stimulation of the hyoepiglotticus muscle was divided by length of the aditus laryngis during breathing while resting to calculate the percentage change in dorsoventral length. The circumference and area of the aditus laryngis were then highlighted and measured, and the area of the aditus laryngis was determined by use of a graphics software program. Area of the aditus laryngis during electrical stimulation of the hyoepiglotticus muscle was divided by the area during breathing while resting to calculate the percentage increase in area with muscle stimulation.

Data analysis—Mean electrical activity was measured during inspiration and expiration for 10 consecutive breaths at each speed. Tonic activity also was measured. Each data point was equivalent to the mean value for 10 consecutive breaths. Respiratory frequency was determined by counting breaths for 15 seconds at each speed. Mean electrical activity was analyzed by use of a 1-way ANOVA on ranks with speed as the main factor. Post hoc comparisons were made by use of the Student-Newman-Keuls test. Timing of muscle activity was determined by comparing the nasopharyngeal pressure waveform to raw EMG activity. The dorsoventral length and area of the aditus laryngis with and without electrical stimulation of the hyoepiglotticus muscle were compared by use of a paired t test. Significance was defined as P < 0.05.

Results
Electrode placement was successful in all horses, and all horses completed the incremental exercise test. Electrical stimulation of the hyoepiglotticus muscle in standing, unsedated horses did not exceed 15 V, because horses began swallowing when the muscle was stimulated at voltages of 10 to 15 V. In all horses, stimulation of the hyoepiglotticus muscle produced ventral displacement of the epiglottis toward the soft palate. In 3 of 6 horses, conformational changes were evident in the epiglottis during muscle stimulation (Fig 1). Dorsoventral length and area of the aditus laryngis were significantly increased during muscle stimulation, such that length of the aditus laryngis increased by 17 ± 3.3% and area of the aditus laryngis increased by 24 ± 6.1%, compared with values when the hyoepiglotticus muscle was not stimulated.

The hyoepiglotticus muscle had respiratory-related electromyographic EMG activity in all horses. During exercise, the hyoepiglotticus muscle had primarily phasic inspiratory activity that increased significantly with increases in treadmill speed (Fig 2). This phasic inspiratory activity preceded inspiration, as determined on the basis of the pharyngeal pressure tracing, by 70 ± 15 milliseconds at HRmax. Four of 6 horses also had phasic expiratory electromyographic EMG activity, but this did not change as treadmill speed increased. Mean electrical activity of the hyoepiglotticus muscle at HRmax was assigned a value of 1, and mean electrical activities at HRmax75, HRmax90, and HRmax were expressed as ratios of the mean electrical activity at HRmax. As treadmill speed increased to HRmax75, to HRmax90, and then to HRmax, inspiratory-phasic mean electrical activity of the hyoepiglotticus muscle increased to 2.70 ± 0.26, 3.60 ± 0.40, and 4.32 ± 0.47, respectively (Fig 3). Tonic activity of the hyoepiglotticus muscle also increased with increases of treadmill speed. Expressed as a ratio of the value at HRmax, tonic activity of the hyoepiglotticus muscle increased at HRmax75, HRmax90, and HRmax to 1.49 ± 0.36, 1.73 ± 0.40, and 2.01 ± 0.48, respectively (Fig 4). Mean ± SEM respiratory frequency at HRmax, HRmax75, HRmax90, and HRmax were 84 ± 9.5, 88 ± 7.5, 83 ± 3.3, and 116.0 ± 5 breaths/min, respectively.

Discussion
Analysis of the findings in the study reported here documented that the hyoepiglotticus muscle had respiratory-related electromyographic activity in horses and that phasic inspiratory and tonic EMG activity increased with increases in treadmill speed and breathing effort. Furthermore, electrical stimulation of the hyoepiglotticus muscle depressed the epiglottis ventrally against the soft palate and enlarged the aditus

Figure 1—Endoscopic images of the larynx of a horse before (A) and during (B) electrical stimulation of the hyoepiglotticus muscle. Notice the depressed, concave appearance of the epiglottis during stimulation, compared with before stimulation.

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laryngis in all horses. On the basis of these results, we concluded that the hyoepiglotticus muscle is likely involved with dilating the airway in horses, which functions to enlarge the aditus laryngis.

Electrical stimulation of the hyoepiglotticus muscle in standing, unsedated horses resulted in an increase in the dorsoventral length of the aditus laryngis. Resistance of the airway to airflow is inversely proportional to the radius of the airway raised to the fourth power. Dilating the airway by increasing the size of the aditus laryngis would likely result in decreased resistance to airflow in exercising horses. Such a decrease in airway resistance would be advantageous to an exercising horse, which has to accommodate an almost 10-fold increase in minute ventilation.

Because horses are obligate nose breathers that cannot breathe orally, they must rely on contraction of muscles that dilate the nares, nasopharynx, and larynx, such as the hyoepiglotticus muscle, to enlarge the airway as minute ventilation increases.

Electrical stimulation of the hyoepiglotticus muscle was used in the study reported here to mimic the effect of muscle contraction on the position and conformation of the epiglottis. Although electrical stimulation in a standing horse may provide information about actions of the hyoepiglotticus muscle, it differs from naturally occurring muscle contractions in an exercising horse and may not approach the magnitude of action of physiologic muscle contraction, resulting in differing effects on epiglottic position. Also, many muscles in this region of the airway contract synchronously during exercise. This combined activity may have differing effects on epiglottic position within the airway. Our attempt to provide quantitative evidence of the effect of stimulation of the hyoepiglotticus muscle on the size of the aditus laryngis was fraught with potential error. Measurements were made on endoscopic images obtained from unsedated horses, with attempts made to standardize the position of the endoscope, arytenoid abduction, and laryngeal size. Although the position of the epiglottis changed with stimulation of the hyoepiglotticus muscle, the actual effect of muscle contraction on size of the aditus laryngis is unknown.

Electromyographic activity of the hyoepiglotticus muscle was synchronous with respiration, and its activity increased significantly with breathing intensity, principally because phasic inspiratory and tonic activity increased. Inspiratory EMG activity preceded the inspiratory decrease in nasopharyngeal pressure. A similar time sequence has been reported in dogs. In dogs, recruitment of the hyoepiglotticus muscle during increased chemical drive results in disengagement of the epiglottis and soft palate such that the epiglottis is depressed against the floor of the oropharynx, permitting oral ventilation. A clinically normal horse will not breathe through its mouth, so recruitment of phasic and tonic hyoepiglotticus EMG activity as treadmill speed increased suggests that the epiglottis is held ventrally against the soft palate during inspiration and expiration. Increased muscle activity is probably necessary to maintain epiglottis position during increased breathing intensity. Factors that drive increased muscle activity during exercise are complex. They include chemical stimuli such as hypercapnia, inputs from mechanoreceptors in limb joints, inputs from sensory receptors in the airway, and increasing central motor drive. These factors
act in concert to enhance excitability of motor neurons in the airway such that the effect on various afferent inputs is amplified by locomotor-linked cortical influences.

In addition to potentially dilating the aditus laryngis, contraction of the hyoepiglotticus muscle stabilizes the epiglottis during inspiration, preventing its prolapse through the aditus laryngis. Retroversion of the epiglottis into the aditus laryngis has been reported²¹ clinically in exercising horses and has been created experimentally,²² by anesthetizing the hypoglossal nerves and geniohyoid muscles. Blockade of these nerves creates dysfunction of the hypoglossal muscles, which suggests that the clinical problem is attributable to paresis of this muscle.

Endoscopic examination of the airway is frequently performed in horses that have exercise intolerance, and it also is part of many pre-sale examinations. During endoscopic examination, veterinarians sometimes describe a condition known as dynamic epiglottis hypoplasia or epiglottic flaccidity because of the fact the epiglottis appears smaller than normal during nasal occlusion or exercise. During the study reported here, we noticed that electrical stimulation of the hyoepiglotticus muscle caused conformational changes in the epiglottis of 3 of 6 horses. As the epiglottis pressed ventrally against the soft palate, its edges rolled slightly inward, and the epiglottis developed a concave shape similar to that described as dynamic epiglottis in inward, and the epiglottis developed a concave shape similar to that described as dynamic epiglottis hypoplasia or epiglottic flaccidity because of the fact the epiglottis appears smaller than normal during nasal occlusion or exercise. During the study reported here, we noticed that electrical stimulation of the hypoglossal and glossopharyngeal nerves creates dysfunction of the hyoepiglotticus muscle.

During the study reported here, we noticed that electrical stimulation of the hyoepiglotticus muscle caused conformational changes in the epiglottis of 3 of 6 horses. As the epiglottis pressed ventrally against the soft palate, its edges rolled slightly inward, and the epiglottis developed a concave shape similar to that described as dynamic epiglottis hypoplasia or epiglottic flaccidity.²² Possibly, the dynamic changes in the appearance of the epiglottis during nasal occlusion or exercise may simply be a result of the normal physiologic action of the hypoglossal muscles and not a conformational abnormality of the epiglottis. In support of this theory, a large retrospective study,²³ of endoscopic findings in horses examined before sale did not find a significant association between apparent epiglottic abnormalities and racing performance.

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