Electromyographic activity of the hyoepiglottic 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 during intense breathing efforts. The hyoepiglotticus muscle may be an important muscle for dilating the airway in horses, and contraction of the hyoepiglotticus muscle may induce conformational changes in the epiglottis. (Am J Vet Res 2002;63:1617–1621)

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 basihyoid 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 hyoepiglotticus muscle is an important muscle for dilating the respiratory passages in horses. It functions to increase the size of the epiglottis 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 for 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 plotted against treadmill speed, and speeds corresponding to HRmax50, HRmax75, HRmax90, and 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 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 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 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 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 throat was prepared for aseptic surgery.

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 hyoepiglotticus 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 sternocervical muscle.

Experimental protocol—The day after electrode placement, electrical stimulation of the hyoepiglotticus 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 hyoepiglotticus 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 naris 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 HRmax90. Nasopharyngeal pressure and electromyography (EMG) signals were recorded throughout the experiment on a computer and a physiologic recorder. The electrode was removed from the hyoepiglotticus 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 hyoepiglotticus muscle was related to nasopharyngeal pressure and was measured by use of the raw EMG signal. The EMG lead-time for the hyoepiglotticus 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 hyoepiglotticus 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 HRmax50 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 HRmax50. 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 HRmax50, 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 HRmax50, 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.
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 mus-
cle in standing, unsedated horses resulted in an
increase in the dorsoventral length of the aditus laryn-
gis. Resistance of the airway to airflow is inversely pro-
portional to the radius of the airway raised to the
fourth power.\textsuperscript{10,12} 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 advan-
tageous to an exercising horse, which has to accommo-
date an almost 10-fold increase in minute ventilation.\textsuperscript{13}
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 mus-
cle was used in the study reported here to mimic the
effect of muscle contraction on the position and con-
formation of the epiglottis. Although electrical stimu-
lation 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 synchro-
nously 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 endo-
sopic images obtained from unsedated horses, with
attempts made to standardize the position of the endo-
scope, 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 laryn-
gis is unknown.

Electromyographic activity of the hyoepiglotti-
cus 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.\textsuperscript{7} 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.\textsuperscript{14} A clin-
ically 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 com-
plex. They include chemical stimuli such as hyper-
capnia, inputs from mechanoreceptors in limb
joints, inputs from sensory receptors in the airway,
and increasing central motor drive.\textsuperscript{14} These factors

\begin{figure}
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\includegraphics[width=0.8\textwidth]{figure2.png}
\caption{Recording of raw and moving time-averaged (MTA) electromyographic (EMG) activity of the hyoepiglotticus muscle and nasopharyngeal pressure at treadmill speeds corresponding to 50\% of maximum heart rate (HR\textsubscript{max50}), 75\% of maximum heart rate (HR\textsubscript{max75}), 90\% of maximum heart rate (HR\textsubscript{max90}), and maximum heart rate (HR\textsubscript{max}). Notice that EMG activity increased with treadmill speed and that peak activity occurred during inspiration (I). E = Expiration.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure3.png}
\caption{Phasic inspiratory EMG activity of the hyoepiglotticus muscle in 6 horses during exercise on a treadmill at various speeds. Circles represent values for each horse, and triangles represent mean values for all 6 horses.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure4.png}
\caption{Tonic EMG activity of the hyoepiglotticus muscle in 6 horses during exercise on a treadmill at various speeds. Circles represent values for each horse, and diamonds represent mean values for all 6 horses.}
\end{figure}
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 reported clinically in exercising horses and has been created experimentally by anesthetizing the hypoglossal nerves and geniohyoid muscles. Blockade of these nerves creates dysfunction of the hypoeptic mucosal muscle, 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 presale examinations. During endoscopic examination, veterinarians sometimes describe a condition known as dynamic epiglottis hypoplasia or epiglottic fllaccidity 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 hypoeptic mucosal 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 horses. As the epiglottis pressed ventrally caused conformational changes in 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 fllaccidity 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 hypoeptic mucosal 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 fllaccidity. 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 hypoeptic mucosal muscle and not a conformational abnormality of the epiglottis. In support of this theory, a large retrospective study of endoscopic findings in horses examined before sale did not find a significant association between apparent epiglottic abnormalities and racing performance.

References