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EFFECT OF STYLOPHARYNGEUS MUSCLE ACTIVITY ON DORSAL NASOPHARYNGEAL FUNCTION IN EXERCISING HORSES

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CAROLINE DOMINIQUE TESSIER

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EFFECT OF STYLOPHARYNGEUS MUSCLE ACTIVITY ON DORSAL NASOPHARYNGEAL FUNCTION IN EXERCISING HORSES

By

Caroline Dominique Tessier

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ABSTRACT

EFFECT OF STYLOPHARYNGEUS MUSCLE ACTIVITY ON DORSAL NASOPHARYNGEAL FUNCTION IN EXERCISING HORSES

By

Caroline Dominique Tessier

Nasopharyngeal collapse has been clinically observed in horses as a cause of upper respiratory obstruction and exercise intolerance, but the etiology of this condition is unknown. The stylopharyngeus muscle, innervated by the glossopharyngeal nerve, is an important pharyngeal dilating muscle in other species, and dysfunction of this muscle results in nasopharyngeal collapse. Six horses were exercised at speeds corresponding to 50%, 75%, and 100% of HR_{max} on the treadmill while upper airway pressures and respiratory frequencies were measured before and after induction of stylopharyngeus muscle dysfunction by bilateral anesthesia of the glossopharyngeal nerve. Next, the electromyographic activity of the stylopharyngeus muscle was recorded in six normal horses performing the same exercise protocol. Bilateral glossopharyngeal nerve block caused stylopharyngeus muscle dysfunction and dorsal nasopharyngeal collapse in all horses. The stylopharyngeus muscle exhibited peak phasic inspiratory activity that increased from 2.5 to 13.6 arbitary units, or 556%, as treadmill speed increased from HR_{max50} to HR_{max}. Therefore, the stylopharyngeus muscle is an important nasopharyngeal dilator and stylopharyngeus dysfunction may be implicated in clinical nasopharyngeal collapse in athletic horses.

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INTRODUCTION

Poor performance in the equine athlete is a problem with multiple facets. However, upper airway disorders seem to have an important role to play (Martin 2000). Since the advent of high-speed treadmill examination, we have been able to recognize a large number of upper airway disorders present only during exercise and therefore high-speed treadmill examination has been recommended to evaluate horses presenting for poor performance with or without respiratory noise (Lumsden 1995). Dynamic upper airway obstructions occur in horses affected by a certain number of disorders such as dorsal displacement of the soft palate, pharyngeal collapse, axial deviation of the aryepiglottic folds, epiglottic entrapment, epiglottic retroversion (Martin 2000). Because of the dynamic and multifactorial nature of these disorders, they are difficult to diagnose and treat effectively. Furthermore, the pathogenesis of dynamic upper airway obstruction remains enigmatic in some diseases such as nasopharyngeal collapse.

Nasopharyngeal collapse in horses

Nasopharyngeal collapse is a disease observed in adult horses that causes upper airway obstruction, exercise intolerance and abnormal upper respiratory noise.

While a few horses affected by nasopharyngeal collapse will show signs of upper airway obstruction or abnormal noise at rest (Smith 1994), the majority of them will only exhibit clinical signs during exercise, making the condition difficult to diagnose. Poor performance and abnormal respiratory noise are some of the owner's common complaints.

The features accompanying this disease are dorsal pharyngeal collapse and collapse of the lateral walls of the nasopharynx. Dorsal pharyngeal collapse can be uni- or bilateral (Smith 1994).

Use of high-speed treadmill endoscopy as a diagnostic tool has improved our recognition of the disease. Reported prevalence rates vary from 3 to 29% (Kannegieter and Dore 1995; Dart 2001; Martin 2000; Durando 2002). In one study, 40 out of 256 horses presented for poor performance had pharyngeal collapse. Thirty of 40 horses with pharyngeal collapse had abnormal upper airway noise that was inspiratory and best heard early or late in the exercise cycle. Collapse of the pharyngeal walls obstructed part or most of the tracheal opening (Martin 2000). Pharyngeal collapse can also be associated with other lesions of the upper respiratory tract. In 92 horses diagnosed with dorsal displacement of the soft palate (DDSP) during treadmill videoendoscopy, 11/92 horses had associated dynamic pharyngeal collapse (Parente 2002). In another retrospective study of 54 horses diagnosed with upper respiratory tract abnormalities during treadmill videoendoscopy, 16 had pharyngeal collapse alone. In this study, pharyngeal collapse was the most represented abnormality related to the presence of abnormal blood gases (decreased PaO2 and/or increased PaCO2) (Durando 2002). Like many other dynamic upper airway disorders, the cause of this disease in horses has not been elucidated and there is no effective treatment available.

Anatomy of the nasopharynx and the pharyngeal musculature in horses

In other species, the action of several groups of muscles, including muscles of the tongue, hyoid apparatus and nasopharynx, is responsible for stiffening and dilating the nasopharynx (Feroah 2000; Kuna 2001; Kuna and Vanoye 1999; Kuna *et al.*1997). These same muscles are also important during deglutition (Kuna and Vanoye 1999). Knowledge of the anatomy of the nasopharynx and the relationships between different muscles is therefore essential to understand pathophysiologic mechanisms responsible for dynamic upper airway disorders.

The pharynx is a musculo-membranous sac that shares digestive and respiratory functions. Horses have no communication between their oro- and nasopharynx because their soft palate contacts the larynx. They are therefore obligate nasal breathers. The pharynx is a funnel-shaped chamber contained between the base of the skull and first couple of cervical vertebrae dorsally, the larynx ventrally, and the pterygoid muscles, the mandible and the dorsal part of the hyoid apparatus laterally. The nasopharynx is about 15cm long and its long axis is directed downward and backward. It is attached by its muscles to the palatine, pterygoid, and hyoid bones, and to the cricoid and thyroid cartilages of the larynx. However, the nasopharynx is not directly supported by bony structures and is therefore susceptible to large amounts of deformation by the negative pressures applied during inhalation.

Its principal relations are: dorsally, to the base of the cranium and guttural pouches; ventrally, to the larynx and soft palate, the latter extending caudally from the hard palate to the base of the larynx and forming the floor of the nasopharynx, and laterally, to the medial pterygoid muscle, the great cornu of the hyoid bone, the external carotid and external maxillary arteries, the glossopharyngeal, anterior laryngeal, and hypoglossal nerves, the mandibular salivary gland, and the parapharyngeal lymph glands (Sisson 1976).

Two groups of muscles will modify the shape and diameter of the pharyngeal opening: intrinsic pharyngeal muscles that form the walls of the nasopharynx and extrinsic pharyngeal muscles which action will also deform the nasopharynx. The intrinsic muscle group is composed of: cricopharyngeus, hyopharyngeus, thyropharyngeus, palatopharyngeus, pterigopharyngeus and stylopharyngeus. These muscles are responsible for constriction and dilation of the nasopharynx (Sisson 1976). The cricopharyngeus muscle originates on the lateral aspect of the cricoid cartilage and attaches on the median raphe. The hyopharyngeus muscle attaches on the hyoid bone and ends at the median raphe, and the thyropharyngeus muscle originates on the lateral lamina of the thyroid cartilage and inserts on the median raphe. These three muscles are pharyngeal constrictors and their shortening depresses the pharyngeal walls down and inward. The palato-pharyngeus arises on the aponeurosis of the soft palate and inserts in part into the upper edge of the thyroid cartilage and in part on the median raphe. The pterygo-pharyngeus muscle lies on the anterior part of the lateral wall of the pharynx. Both of these muscles act to shorten the pharynx and to draw the larynx and esophagus toward the root of the tongue in swallowing. The stylopharyngeus muscle arises from the medial surface of the great cornu of the stylohyoid bone, passes ventromedially and enters the wall of the pharynx by passing between the pterygo-pharyngeus and the palato-pharyngeus muscles. Its fibers radiate, many bundles passing forward, others inward or backward beneath the hyopharyngeus. It raises and dilates the pharynx to receive the bolus in swallowing (Sisson and Grossman 1975). In people, it is known that contraction of the stylopharyngeus muscle dilates the dorsal part of the nasopharynx (Kuna and Vanoye 1999).

The extrinsic muscles are part of the hyoid apparatus: geniohyoid, genioglossus, sternohyoid, sternothyroid and hyoepiglotticus muscles. The hyoid apparatus is attached to the petrous temporal bone and supports the root of the tongue, pharynx and larynx. The basihyoid bone is closely related to the base of the tongue via its attachments to the genioglossus and hyoglossus muscles. It articulates with the short ceratohyoid bone located just cranial to it. The latter joints the stylohyoid bone, which extends from the skull cranially. Forward and downward movement of the tongue will therefore enlarge the nasopharynx by extending the ceratohyoid-stylohyoid articulation, thus enlarging the dorsoventral diameter of the nasopharynx.

Role of the pharyngeal dilator muscles in people and laboratory animals

Because of the increased recognition of sleep disorders in people, and sleep apneas in particular, many studies have been conducted in man and laboratory animals in order to understand the role of intrinsic pharyngeal muscles, in particular those responsible for dilation of the nasopharynx. Among intrinsic upper airway muscles, the pharyngeal dilators responsible for enlarging the nasopharynx in response to different stimuli such as upper airway negative pressures. The stylopharyngeus muscle is part of this group of muscles. In normal awake goats, the stylopharyngeus muscle was found to exhibit tonic expiratory and phasic inspiratory activity, whereas the pharyngeal constrictors were active during expiration. This duality of action allows the nasopharynx to adapt its size and shape to the airflow and resist collapse when large negative pressures are applied to the upper airway (Feroah 2000). Furthermore, when undergoing slow wave (SWS) and rapid eye movement (REM) sleep, the same animals showed a decrease in

stylopharyngeus muscle activity from awake to SWS and from SWS to REM sleep, indicating a possible involvement of stylopharyngeus muscle in sleep apnea (Feroah 2001a). In those same conditions, when negative pressure was applied, the stylopharyngeus muscle responded by increasing its activity both in awake and SWS, but the activity did not change in REM sleep compared to controls. These data indicate that the stylopharyngeus muscle does participate in the compensatory mechanisms that adjust to increasing loads of negative pressure (Feroah 2001b). In one study performed on anesthetized rabbits, it was found that another pharyngeal dilator, the genioglossus muscle, responds to augmented upper airway negative pressures by increasing its activity by several folds. Section of the motor output to the genioglossus muscle abolished this response. The pharyngeal dilator muscles have a role in compensation for added inspiratory load and the activation of these muscles facilitates the load compensating function of diaphragmatic muscles by decreasing airway resistance (Aleksandrova 2002). Furthermore, in a study conducted in man, the investigators found that sudden upper airway negative pressure applied at the end of expiration activates the genioglossus muscle. They concluded that a reflex pathway was present that resulted in pharyngeal dilator muscle activation in the presence of intrapharyngeal forces that otherwise may narrow or close the pharynx (Horner 1991).

Role of the stylopharyngeus muscle

The stylopharyngeus muscle is innervated by the glossopharyngeal nerve, which is a mixed nerve containing both motor and sensory fibers (Sisson 1976). The latter constitute the bulk of the nerve and include those that mediate the sense of taste. The

glossopharyngeal nerve, after exiting the cranial cavity, curves ventrad and rostrad over the guttural pouch and caudal to the thyrohyoid bone, crosses the deep face of the external carotid artery and divides into pharyngeal and lingual branches. It also gives off collateral branches, which are the tympanic nerve, the lesser petrosal nerve, and a branch to the carotid sinus. The very small branch of the glossopharyngeal nerve to the stylopharyngeus muscle arises from the dorsal border of the nerve. The pharyngeal branch runs rostrad across the deep face of the stylohyoid bone and joins the pharyngeal branches of the vagus and sympathetic filaments in forming the pharyngeal plexus; several branches pass to the muscles and mucous membranes of the pharynx. The lingual branch is the continuation of the trunk and runs along the caudal border of the stylohyoid bone rostral to the linguofacial trunk before it dives under the hyoglossus muscle (Sisson and Grossman 1976, Ozveren 2003). This cranial nerve has mostly sensory fibers, but provides motor innervation to only one skeletal muscle, the stylopharyngeus. Sensory fibers are distributed in the mucous membranes of the pharyngeal walls, soft palate, isthmus faucium or the mucosa of the oral pharynx, and tonsils (Sisson 1976).

The role of cranial nerves, glossopharyngeal nerve in particular, has been extensively studied in a human model for obstructive sleep apnea. Results show that electrical stimulation of the glossopharyngeal nerve causes pharyngeal dilation in cats, by stimulating contraction of the stylopharyngeus muscle (Iizuka 2001; Kuna 2001). Furthermore, in adult rats, the glossopharyngeal nerve exhibits respiratory burst activity that starts in the late expiratory phase and terminates at the end of the inspiratory phase (Frugiere and Barillot 1994). Afferent branches of the glossopharyngeal nerve have

therefore been implicated in the pathogenesis of dynamic collapse of the upper airway in other species.

The upper airway dilating muscles are activated in sequence

In many species, the dilating muscles of the nasopharynx are recruited in a coordinated sequence within the respiratory pattern in order to stabilize the nasopharynx when negative upper airway pressures are produced. In general, phasic activation of upper airway muscles precedes inspiratory activity of the diaphragm, and augmented upper airway pressures increase this amount of pre-activation (Van Lunteren 1984). Negative upper airway pressure has also been shown to reflexly augment phasic inspiratory and tonic electromyographic (EMG) activity in many upper airway dilator muscles and to play an important role in regulating EMG activity of these muscles during breathing in animals and humans (Van Lunteren 1984, Horner 1991, Van Der Touw 1994). In awake goats, the stylopharyngeus muscle has been shown to display phasic inspiratory discharge before the onset of diaphragmatic activity and its activity peaks before peak diaphragmatic activity. A tonic discharge was also observed throughout respiration, except at the beginning of expiration (Feroah 2000). Reflex augmentation of upper airway muscle activity may have functional significance in the maintenance of upper airway patency. It potentially prevents upper airway collapse in the face of negative pressure oscillations in the upper airway or facilitates recovery when large negative pressure changes are produced by inspiratory efforts against an obstructed airway (Mathew 1984).

Influence of upper airway receptors on recruitment of upper airway muscles

Glossopharyngeal afferent fibers found beneath the nasopharyngeal mucosa mediate the sniff-like aspiration reflex elicited by mechanical deformation of the nasopharyngeal mucosa (Sant'Ambrogio *et al.* 1995). Some of the receptors within the nasopharynx innervated by the glossopharyngeal nerve respond to positive and negative pressure, but the pharynx is not an important site for reflex generation and stimulation of upper airway patency maintaining muscles. (Horner *et al.* 1994) Sectioning the glossopharyngeal nerve caused increased rather than decreased response to pressure with in the nasopharynx (Mathew *et al.* 1988).

The laryngeal mucosa has an abundant supply of sensory receptors controlling a complex pattern of respiratory reflexes that influence the patency of the upper airway and the breathing pattern. Three types of laryngeal receptors have been identified, responding specifically to transmural pressure, airflow and contraction of the laryngeal muscles (Sant'Ambrogio 1985). Laryngeal pressure receptors respond to either negative or positive pressure and a few respond to both. Reflex alterations in the breathing pattern and upper airway muscle activity during upper airway pressure changes are mediated by such receptors. (Mathew 1984). The laryngeal afferents follow 3 routes: the internal branch of the superior laryngeal nerve (SLN), the external branch of the same nerve, and the recurrent laryngeal nerve (Mathew and Sant'Ambrogio 1988). Section of the superior laryngeal nerve has been shown to decrease the EMG activity of the pharyngeal dilator muscles in response to upper airway negative pressure and to decrease the level of

negative pressures required to elicit upper airway collapse (Horner 1991). Furthermore, the reflex activation of upper airway dilators by increase of negative pressures in the upper airway is abolished by topical anesthesia of the laryngeal mucosa (Van Lunteren 1984). Desensitization of the laryngeal mucosa has been shown to induce nasopharyngeal collapse and upper airway obstruction in normal exercising horses (Holcombe 2001).

The laryngeal mucosa has been shown to contain multiple mechanoreceptors involved in the reflex pathways involving recruitment of the upper airway muscles (Sant'Ambrogio 1983). The stimulus capable of eliciting such reflex is the presence of subatmospheric pressure in the upper airway. Laryngeal receptors are believed to be the principal transducing elements that initiate this response (Kuna 1991). In human studies, it has been shown that upper airway dilator muscles can be activated throughout inspiration via ongoing upper airway mechanoreceptor reflexes. Such a feedback mechanism protects upper airway patency within each breath in awake humans (Akahoshi 2001). These receptors are thought to respond to negative pressures and their activity increases in face of upper airway obstruction, therefore promoting upper airway patency (Sant'Ambrogio 1983).

We have seen that upper airway dilating muscles and the stylopharyngeus muscle in particular play an important role in supporting the nasopharynx during inspiration in people and laboratory animals. These muscles are recruited in sequence during the breathing effort to maintain nasophaynrgeal patency when negative inspiratory pressures are applied to the nasopharynx. In horses, little is known about the pathogenesis of

nasopharyngeal collapse and it seemed likely that the stylopharyngeus muscle would be involved in this phenomenon. Furthermore, it is yet unknown how the upper airway dilating muscles are recruited and activated during exercise in horses, information which could be very useful in the understanding of dynamic upper airway disorders.

Aim of the thesis

The aim of this thesis is to investigate the role of the stylopharyngeus muscle in dorsal nasopharyngeal function in exercising horses and attempt to explain the pathophysiology of dorsal nasopharyngeal collapse in exercising horses.

Our first hypothesis was that bilateral anesthesia of the glossopharyngeal nerves causes stylopharyngeus muscle dysfunction and subsequent dorsal pharyngeal collapse and upper airway obstruction in exercising horses. I determined if bilateral local anesthesia of the glossopharyngeal nerve resulted in stylopharyngeus muscle dysfunction and subsequent dorsal pharyngeal collapse. The second hypothesis was that the stylopharyngeus muscle has inspiratory activity that increases with exercise intensity. I then aimed to correlate the electromyographic activity of the stylopharyngeus muscle to the breathing pattern in horses exercising on the treadmill.

CHAPTER 1

Effect of stylopharyngeus muscle dysfunction on the nasopharynx in exercising horses

Reasons for performing the study: To determine the function of the stylopharyngeus muscle on nasopharyngeal function in horses and to investigate a potential cause of dorsal nasopharyngeal collapse in horses.

Objective: To determine the effect of bilateral glossopharyngeal nerve block and stylopharyngeus muscle dysfunction on nasopharyngeal function and airway pressures in exercising horses.

Methods: Endoscopic examinations were performed on horses at rest and while the horses were running on a treadmill at speeds corresponding to HR_{max50}, HR_{max75}, and HR_{max} while upper airway pressures were measured, with and without bilateral glossopharyngeal nerve block.

Results: Bilateral glossopharyngeal nerve block caused stylopharyngeus muscle dysfunction and dorsal nasopharyngeal collapse in all horses. Peak inspiratory upper airway pressure was significantly ($P = 0.0069$) more negative at all speeds and respiratory frequency was lower ($P = 0.017$) in horses with bilateral glossopharyngeal nerve block and stylopharyngeus muscle dysfunction compared to control values.

Conclusions: Bilateral glossopharyngeal nerve anesthesia produced stylopharyngeus muscle dysfunction, dorsal pharyngeal collapse, and airway obstruction in all horses.

Potential relevance: The stylopharyngeus muscle is likely an important nasopharyngeal dilating muscle in horses and dysfunction of this muscle may be implicated in clinical cases of dorsal nasopharyngeal collapse.

Introduction

Nasopharyngeal collapse has been clinically observed in horses as a potential cause of exercise intolerance and upper respiratory noise (Kanniegeter and Dore 1995). Ventral displacement of the roof of the nasopharynx and/or axial displacement of the lateral walls of the nasopharynx are common features observed in exercising horses with this disease (Kanniegeter and Dore 1995). Unfortunately, the cause of this obstructive disease is unknown. No treatment is available for this condition and affected horses are often retired from performance.

The action of several groups of muscles is responsible for stiffening and dilating the nasopharynx (Feroah 2000; Kuna 2001; Kuna and Vanoye 1999; Kuna *et al.*1997). These same muscles are also important during deglutition (Kuna and Vanoye 1999). This dichotomy of action, contracting the pharyngeal walls during swallowing and dilating the airway during breathing, seems contradictory. However, these muscles are uniquely situated to perform both activities, because the pharynx is a conduit for both food and air. The dorsal or superior pharyngeal constrictor muscles contract during swallowing, forming a sphincter and moving the food bolus into the esophagus. These same muscles have tonic activity during respiration such that they form a rigid pharyngeal roof during breathing (Kuna 2001; Kuna and Vanoye 1999). Contraction of the stylopharyngeus muscle dilates the dorsal part of the nasopharynx in people (Kuna and Vanoye 1999). In horses, this muscle originates on the axial aspect of the distal portion of the stylohyoid bone. It courses rostroventrally and ramifies in the wall of the dorsal nasopharynx, by passing between the pterygopharyngeus and palatopharyngeus muscles (Fig 1). In

horses, contraction of the stylopharyngeus muscles raises the roof of the pharynx and dilates the pharynx to receive the bolus during swallowing (Sisson 1976). In a similar manner, we hypothesized that during breathing, contraction of the stylopharyngeus muscle pulls the nasopharyngeal wall dorsally thereby supporting the dorsal wall of the nasopharynx and preventing dynamic collapse of this area during inspiration.

The glossopharyngeal nerve provides motor innervation to the stylopharyngeus muscle. This cranial nerve has mostly sensory fibers, but provides exclusive motor innervation to only one skeletal muscle, the stylopharyngeus. Electrical stimulation of the glossopharyngeal nerve causes pharyngeal dilation in cats, by stimulating contraction of the stylopharyngeus muscle (Iizuka 2001; Kuna 2001). Therefore, the purpose of our investigation was to determine if bilateral glossopharyngeal nerve blockade and the resulting stylopharyngeus dysfunction would cause collapse of the dorsal nasopharynx in exercising horses and if the induced collapse would result in airway obstruction.

Material and Methods

Horses—Six horses (4 Standardbreds and 2 mixed-breeds, 2 geldings and 4 mares, 4 to 13 years old, weighing between 400 and 427kg) were used in this experiment, which was approved by the All-University Committee for Animal Use and Care at Michigan State University. These horses had normal upper airway function based on physical examinations and endoscopic examinations of the larynx and nasopharynx at rest and during high-speed treadmill exercise.

Training procedure—All horses were trained to run on the treadmill prior to onset of the study. Maximum heart rate (HR_{max}) was determined during an incremental exercise test that consisted of a warm-up period of 3 minutes at a speed of 4 m/s, 2 minutes at a speed of 6 m/s, and 1 minute at increasing speeds of 8, 10, 11, 12, and 13m/s or until the horses became fatigued and were unable to maintain their position on the treadmill despite humane encouragement. During this test, heart rate was recorded by use of a telemetry system.[1] The speeds corresponding to 50% of maximum heart rate (HR_{max50}) and 75% of maximum heart rate (HR_{max75}) were interpolated from this data.

Glossopharyngeal nerve anesthesia - An injection apparatus was made using a 12 ml syringe attached to a 30 cm length of polyethylene tubing (I.D. 1.19 mm, O.D. 1.7 mm) with a 22 gauge needle without its hub attached to the end of the tubing. The tubing was inserted through a sclerotherapy needle[2] apparatus after the inner tubing of this apparatus was removed. The apparatus was then passed through the biopsy channel of the endoscope. The apparatus was used to inject local anesthetic around the glossopharyngeal nerve, as it coursed rostroventrally through the medial compartment of the guttural pouch. The injection site was rostral to the external carotid artery. This placement was chosen because the hypoglossal nerve dives deep and caudal to the external carotid artery. In this location, the glossopharyngeal nerve is isolated such that the nerve block would be specific for the glossopharyngeal nerve.

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[1] Digital telemetry system, MI 403A. Hewlett Packard, Palo Alto, California, USA

[2] Millrose Laboratories, Mentor, Ohio, USA

Horses were restrained in a set of stocks and a lip twitch was applied. The video-endoscope was passed through the right naris, into the right guttural pouch. The glossopharyngeal nerve was identified coursing across the external carotid artery within the medial compartment of the guttural pouch. Perineural anesthesia was performed by injecting 0.5 to 1 cc of 2% mepivicaine hydrochloride[3] beneath the guttural pouch mucosa, directly over the glossopharyngeal nerve, just rostral to the external carotid artery. Anesthesia of the left glossopharyngeal nerve was achieved in a similar manner.

Upper airway pressure measurements - Upper airway pressures were measured in horses running on the treadmill. Using endoscopic guidance, a 150-cm polyethylene (inner diameter, 2.15 mm; outer diameter, 3.25 mm) catheter with 6 holes on the side of the catheter beginning a distance of 26 mm from the sealed tip was passed through the right nostril and through the laryngeal opening into the trachea (Nielan *et al.* 1992). The tip of this catheter was placed approximately at the junction of the proximal and middle thirds of the cervical portion of the tracheal. The catheter was connected to a differential pressure transducer[4] and upper airway pressures were recorded by use of a chart recorder[5]. The differential pressure transducer was calibrated by use of a water manometer before each experiment. Peak inspiratory and expiratory tracheal pressure and respiratory frequency were determined from the pressure recorder tracings. Ten consecutive breaths were averaged to determine each data point.

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[3] The Upjohn Company, Kalamazoo, Michigan, USA

[4] DP-4522, Validyne Engineering Sales, Northridge, California, USA

[5] Dash II, Astromed, West Warwick, Rhode Island, USA

Experimental Design- Horses were fasted for a minimum of 3 hours before the experiment to minimize gastric contents. Twenty minutes after the bilateral glossopharyngeal nerve block was performed, the horse was restrained with a lip twitch and the endoscope was passed through the right nostril. The horse's nares were occluded for 60 second while observing the nasopharynx with the endoscope to assess upper airway function. The endoscopic examination was recorded. To test if the glossopharyngeal nerves had been anesthetized, a biopsy instrument was used to probe the dorsal and lateral walls of the nasopharyngeal mucosa and the soft palate. Lack of swallowing or gagging in response to the tactile stimulation indicated successful anesthesia of the glossopharyngeal nerves. With endoscopic guidance, a catheter was placed into the trachea. Horses were then exercised on the treadmill for 3 minutes at 4 m/s and 1 min at the speed corresponding to HR_{max50}. Subsequently, a videoendoscope was placed through the right naris and secured to the halter with rubber tubing. Horses were exercised at HR_{max50}, HR_{max75}, and HR_{max} for 1 min at each speed. Upper airway pressure measurements were recorded throughout the exercise trial. All endoscopic examinations were videotaped so that upper airway function could be assessed. Horses performed the exercise trial twice: once following bilateral glossopharyngeal nerve block and once without the nerve block. The sequence of exercise trials was randomized so that 3 horses performed the trial with anesthesia first, and 3 horses performed the trial first without anesthesia.

Data analysis

Data were analyzed using a two-way analysis of variance, with speed and nerve block as the main factors. Post hoc comparisons, where appropriate, were made using the Student-Newman-Keuls test. A significance level of $P < 0.05$ was selected.

Results

All horses tolerated the glossopharyngeal nerve block well and bilateral glossopharyngeal nerve anesthesia was obtained in every horse, such that none of the horses reacted to tactile stimulation of the dorsal pharynx by gagging or swallowing. All horses completed the exercise protocol. None of the horses showed signs of dysphagia, coughing, or respiratory difficulty following bilateral glossopharyngeal nerve anesthesia.

All horses had normal upper airway function during upper airway endoscopy, both at rest while the nares were occluded and during exercise, without bilateral glossopharyngeal nerve block. In contrast, nasal occlusion at rest caused dorsal pharyngeal collapse in 4 out of 6 horses following bilateral glossopharyngeal nerve anesthesia (Fig 3). Furthermore, the dorsal nasopharynx collapsed in all horses during treadmill exercise (Fig 4). Specifically, during inspiration, the dorsal wall of the nasopharynx collapsed ventrally such that the corniculate processes of the arytenoid cartilages could not be seen. None of the horses displaced their soft palate or collapsed the lateral walls of the nasopharynx. Two horses had endoscopic evidence of only unilateral collapse during resting and treadmill endoscopy. In these horse the dorsal hemi-nasopharynx collapsed ventrally during inhalation, while the contralateral side maintained a normal position (Fig 5). This unilateral collapse was likely due to ineffective glossopharyngeal nerve block on the non-collapsing side. Bilateral glossopharyngeal anesthesia was performed

successfully on these two horses 10 days to 2 weeks later, and only data obtained from successful bilateral blockade protocols was analyzed.

As treadmill speed increased from HR_{max50} to HR_{max75} and from HR_{max75} to HR_{max}, peak inspiratory upper airway pressure became significantly more negative. Also, as speed increased from HR_{max50} to HR_{max}, respiratory frequency increased. There was no significant effect of treadmill speed on peak expiratory upper airway pressure. The main effects of the bilateral glossopharyngeal nerve block were significantly (P=0.0069) more negative peak upper airway inspiratory pressure (Fig. 6) and significantly (P = 0.017) lower respiratory frequency. With bilateral glossopharyngeal nerve block, peak inspiratory upper airway pressures (mean ± SEM) at HR_{max50}, HR_{max75}, and HR_{max} were –21.0 ± 2.67, -32.1 ± 3.41 and –37.4 ± 2.71 cm H_20, respectively, compared with –15.0 ± 0.94, -23.3 ± 1.57, and –30.4 ± 1.50 cm H_20 without the nerve block. Also, respiratory frequency at HR_{max50}, HR_{max75}, and HR_{max} was 63 ± 4.8, 79 ±8.6, and 96 ± 11.8 breaths per minute with the nerve block compared to 71 ± 4.2, 83 ± 9.2, and 99 ± 10 breaths per minute without the nerve block. We were unable to detect a significant effect of bilateral glossopharyngeal nerve block on peak upper airway expiratory pressures.

Discussion

The results of the study reported here documented that dysfunction of the stylopharyngeus muscle, induced by bilateral glossopharyngeal nerve block, resulted in ventral displacement of the dorsal nasopharyngeal wall in exercising horses. Following bilateral glossopharyngeal nerve block, peak inspiratory airway pressures were more

negative at all speeds, suggesting that stylopharyngeus dysfunction and the resulting nasopharyngeal collapse caused airway obstruction. Therefore, based upon this information, we conclude that the stylopharyngeus muscle is an important nasopharyngeal dilating and stabilizing muscle in horses.

Bilateral glossopharyngeal nerve block resulted in dynamic collapse of the dorsal nasopharynx, both at rest when the horses' nares were occluded, and while the horses ran on the treadmill. Specifically, the dorsal wall of the nasopharynx collapsed ventrally during inspiration, obscuring the view of the corniculate processes of the arytenoid cartilages and partially obstructing the rima glottidis. Anatomic considerations suggest that this dynamic obstruction was caused by stylopharyngeus muscle dysfunction. The stylopharyngeus muscles insert on the dorsal nasopharyngeal wall, perpendicular to it, such that contraction of the stylopharyngeus muscles raises the wall of the dorsal nasopharynx, expanding and supporting the dorsal nasopharynx, and preventing collapse as pressures within the airway become more negative during inspiration.

Bilateral glossopharyngeal nerve anesthesia also resulted in more negative peak inspiratory airway pressures indicating that the induced nasopharyngeal collapse caused airway obstruction. The average decrease in peak inspiratory pressure was approximately 8 cm H_2O or a 25% decrease or more negative inspiratory pressure. This change in inspiratory pressure was small compared to the change in inspiratory pressure measured in horses with left laryngeal hemiplegia, which approached 30 to 40 cm H_2O. (Tetens *et al.* 1996) Despite this observation, the obstruction resulted in an approximate 25%

decrease in peak inspiratory pressure, suggesting that seemingly mild nasopharyngeal collapse in horses, clinically, may have significant functional consequences.

Interestingly, the horses with the most severe collapse of the dorsal nasopharynx, based on endoscopic examination, did not have the largest changes in peak inspiratory airway pressure: some horses had minor collapse, as viewed endoscopically, with large changes in inspiratory pressures. This might be explained by the fact that while a decrease in nasopharyngeal diameter is easily observed through the endoscope, the length of the obstruction is harder to evaluate. The stylopharyngeus muscle has a broad insertion that is approximately 4 cm long (based upon measurements made in 10 cadaver horse heads). The nasopharyngeal collapse induced by bilateral glossopharyngeal nerve anesthesia obstructed the dorsal portion of the rima glottidis in a sagittal plane but also obstructed the nasopharynx along a length of at least 4 cm, based upon the broad insertion of the stylopharyngeus muscle. This is important because airway resistance is indirectly proportional to the radius but also directly proportional to the length of the affected segment. Therefore, the airway pressure measurements may more accurately describe the degree of airway obstruction in each horse.

The glossopharyngeal nerve is primarily a sensory nerve and provides exclusive motor innervation to the stylopharyngeus muscle (Sisson 1976). The pharyngeal mucosa contains a variety of sensory receptors including pressure and stretch receptors, chemical and mechanical receptors and nocioceptive endings that when triggered, stimulate the swallowing reflex.(Sant'Ambrogio *et al.* 1995) Glossopharyngeal afferent fibers found

beneath the nasopharyngeal mucosa mediate the sniff-like aspiration reflex elicited by mechanical deformation of the nasopharyngeal mucosa.(Sant'Ambrogio *et al.* 1995) Some of the receptors within the nasopharynx innervated by the glossopharyngeal nerve respond to positive and negative pressure, but the pharynx is not an important site for reflex generation and stimulation of upper airway patency maintaining muscles. (Horner *et al.* 1991) Sectioning the glossopharyngeal nerve caused increased rather than decreased response to pressure with in the nasopharynx (Mathew *et al* 1988). Therefore, it is unlikely that the nasopharyngeal collapse created by bilateral glossopharyngeal nerve block was due to decreased sensory activity within the nasopharynx.

In addition to providing motor innervation to the stylopharyngeus muscle, studies in rhesus monkeys, dogs and rats indicate that branches of the glossopharyngeal nerve could also provide some motor fibers to the levator veli palatini, and superior or dorsal pharyngeal constrictor muscles, via fibers entering the pharyngeal plexus and joining fibers from the vagus nerve (Furusawa et al. 1996, Nishio *et al.* 1976, Venker-van Haagen *et al.* 1986). It is unlikely that the dorsal nasopharyngeal collapse created here by bilateral glossopharyngeal nerve block was caused by dysfunction of the levator veli palatini or superior pharyngeal constricting muscles, because the principle motor supply to these muscles is the vagus nerve, which was not anesthetized in this study. As well, we did not appreciate dysphagia in any of the horses following bilateral glossopharyngeal nerve block, which would occur with levator veli palatini and dorsal pharyngeal constricting muscle dysfunction (DeLahunta, 1977; Mayhew 1989).

Glossopharyngeal nerve integrity is traditionally evaluated along with the vagus nerve by testing swallowing function. Interestingly, none of the horses in this study showed clinical signs of dysphagia following bilateral glossopharyngeal nerve anesthesia. The horses never had saliva pooling at the nares or coughed during the exercise trial. Furthermore, approximately 30 min after the end of the exercise trial the horses were given water and hay and monitored closely for 2 hours. No signs of dysphagia were observed in any of the horses in this study. During swallowing, constriction of the pharyngeal lumen, in a peristaltic sequence, results from displacement of the tongue, hyoid bone and larynx, and from contraction of the pharyngeal constrictor and dilator muscles (Bosman *et al.* 1986). It is possible that, rather than causing a complete dysphagia with lack of propulsive activity from the pharynx, bilateral glossopharyngeal nerve block resulted in changes in the sequence or relative timing of swallowing that could not be detected clinically. Similarly, following bilateral glossopharyngeal nerve sectioning in dogs, the animals showed no clinical signs of dysphagia, but swallowing dynamics and sequence was altered, based on fluoroscopic studies (Venker-van Haagen *et al.* 1986).

All horses significantly decreased their respiratory frequencies after glossopharyngeal blockade. The decrease in respiratory frequency was small, approximately 3 to 10 breaths per minute, but it occurred in 5 of 6 of horses at all speeds. We did not measure tidal volume or airflow in this study, and therefore, attempted to explain the decreased respiratory frequency as a technique employed by the horses to compensate for the imposed airway obstruction. In the face of upper airway obstruction caused by the dorsal

nasopharyngeal collapse, the resistance to the airflow through the upper airway likely increased. With increased resistance to breathing inspiratory time is increased, in an attempt to maintain tidal volume, ultimately resulting in decreased respiratory frequency (Daubenspeck 1981). Therefore, the horses modified their breathing technique by lowering respiratory frequency in response to the imposed airway obstruction caused by nasopharyngeal collapse.

CHAPTER 2

Electromyographic activity of the stylopharyngeus muscle in exercising horses.

Summary

Reasons for performing the study: To determine if the stylopharyngeus muscle has respiratory-related activity that increases with exercise intensity in horses.

Objective: To measure the electromyographic activity of the stylopharyngeus muscle in exercising horses and correlate it with the breathing pattern.

Methods: Electromyographic activity of the stylopharyngeus muscle and upper airway pressures were recorded in normal horses running on a treadmill.

Results: The stylopharyngeus muscle has phasic inspiratory activity that increases with speed.

Conclusions: The stylopharyngeus muscle is an upper airway dilating muscle that functions principally during inspiration and its activity increases with exercise intensity.

Potential relevance: The stylopharyngeus muscle is one of a group of upper airway dilating muscles that functions to support structures of the nasopharynx during inspiration.

Introduction

Nasopharyngeal collapse has been clinically observed in horses as a cause of exercise intolerance and upper respiratory noise (Kannegieter 1995). When this disease occurs, collapse of the dorsal wall of the nasopharynx is one of the features observed in horses during treadmill exercise. In horses, little is known about the activity of the pharyngeal muscles in relation to the breathing pattern and the pathways by which they become recruited during respiration to maintain nasopharyngeal patency. In a previous study we documented that anesthesia of the glossopharyngeal nerves and the resulting stylopharyngeus muscle dysfunction caused dorsal pharyngeal collapse and inspiratory upper airway obstruction in normal horses during exercise. We concluded that the stylopharyngeus muscle is an important dilator of the dorsal nasopharynx in horses and that dysfunction of this muscle could be implicated in clinical cases of dorsal pharyngeal collapse (Tessier 2004).

Before and during inspiration, the pharyngeal muscles are recruited and activated in a coordinated sequence in order to stabilize the nasopharynx in response to negative intraluminal pressures applied to the upper airway (Van Lunteren 1988). Upper airway muscles show a very rapid increase in activity that precedes the onset of respiratory pump muscle activation by as much as 200ms (Van Lunteren 1983; Kuna 1991). In other species, the stylopharyngeus muscle has been shown to exhibit respiratory related activity (Feroah 2000). Therefore, we hypothesized that in horses the stylopharyngeus muscle would have inspiratory related activity that would increase with increased breathing intensity induced by increasing levels of exercise.

Material and Methods

Horses—Five Standardbred horses (3 geldings and 2 mares, aged 5-12 years) were used in the experiment, which was approved by the All-University Committee for Animal Use and Care at Michigan State University. The horses were determined to be normal based on physical and endoscopic examinations of the larynx and nasopharynx at rest and during high-speed treadmill exercise. All horses were trained to run on the treadmill prior to the experiment. Maximum heart rate (HR_{max}) was determined by use of a telemetry system[1] and an incremental exercise test. The speeds corresponding to 50% (HR_{max50}) and 75% (HR_{max75}) of maximum heart rate were interpolated from these data.

Electrode placement- Bipolar fine wire electrodes and a ground wires were constructed using Teflon-coated wire[2]. A small amount of resin cement[3] was applied to the end of the electrode. The electrode was placed in the stylopharyngeus muscle through the nasopharyngeal opening of the guttural pouch while the horse was anesthetized. The horse was pre-medicated with xylazine (0.04 mg/kg, IV), anesthesia was induced with ketamine (2.2 mg/kg, IV) and diazepam (0.1 mg/kg, IV) and the horse was positioned in sternal recumbency with its head elevated. Anesthesia was maintained by use of an intravenous infusion of 5% guaifenesin containing 0.05 mg of xylazine/ml and 2.0 mg of ketamine/ml, delivered at 2 ml/kg/h. Twenty gauge needles with the hubs removed were secured to each end of the bipolar electrode. The needle was grasped with the biopsy instrument and pulled into the biopsy channel.

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[1] Digital telemetry system, MI 403A. Hewlett Packard, Palo Alto, California, USA

[2] Teflon-coated wire, No. A5637, Coonerwire, Chatsworth, PA

[3] Resin cement kit, Justi Products, Oxnard, CA

The endoscope was then passed through the right nostril and into the right guttural pouch. The bipolar electrode was positioned into the stylopharyngeus muscle by pushing the needle attached to the electrode wire out of the biopsy channel and through the origin of the stylopharyngeus muscle on the stylohyoid bone, using the biopsy instrument. This procedure was performed twice to seat both segments of the bipolar electrode. The electrode wires exited the guttural pouch and were passed through the nostril using a 14-gauge needle and secured to the horse's muzzle using elastic tape. A ground wire was placed in the subcutaneous space, just over the right sternocephalicus muscle. The following day, muscle function measurements were made while the horses exercised on a treadmill.

Experimental design- A 150cm long catheter[4] (polyethylene tubing, 2.15 mm ID, 3.25 mm OD) with 6 side holes beginning a distance of 8 catheter diameters from the sealed tip, was placed through the left naris and secured to the muzzle with tape. The catheter tip was placed at the level of the nasopharyngeal opening of the guttural pouch in the nasopharynx to measure upper airway pressures so that the EMG activity could be correlated with respiration. Horses were initially exercised on the treadmill for 3 minutes at 4 m/s and 1 min at the speed corresponding to HR_{max50}. Then, the exercise trial consisted of three continuous one-minute segments of exercise at HR_{max50}, HR_{max75}, and HR_{max} respectively. Upper airway pressure measurements and EMG activity were recorded throughout the exercise trial. Peak inspiratory and expiratory pressures and respiratory frequency were determined from the pressure tracings.

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[4] Baxter Scientific Products, McGaw Park, IL

Ten consecutive breaths were averaged to determine each data point. Following the exercise trial, the horses were rested until their heart and respiratory rates had returned to normal.

Upper airway pressure measurements - The nasopharyngeal catheter was connected to a differential pressure transducer[5] and upper airway pressures were recorded by use of a chart recorder[6] used to record the EMG tracings. The differential pressure transducer was calibrated by use of a water manometer to 5, 10, and 20 cm of H_2O before each experiment. The pressure tracing was used to correlate the EMG activity of the stylopharyngeus muscle with the breathing pattern.

EMG measurements- The EMG signals were processed through a sixth-order Butterworth filter (band pass, 50 to 5000 Hz), amplified, rectified, and moving time-averaged with a constant of 100 milliseconds. Both raw EMG and moving time average signals were recorded[7]. Quantification of the EMG was performed by digitization of the moving time-averaged EMG signal using an image acquisition and analysis, frame-grabber software[8].

Data analysis- Respiratory timing was based on the pharyngeal pressures and determined using the EMG signals that were recorded simultaneously with pharyngeal pressure. Mean electrical activity was calculated by dividing the total area under each moving time average waveform above baseline by the duration of the electrical activity. The average

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[5] Dash II, Astromed, West Warwick, Rhode Island, USA

[6] DP-4522, Validyne Engineering Sales, Northridge, California, USA

[7] LabVIEW 5.0, National Instruments Software, Austin, Texas

[8] Scion Image, Scion Corp, Frederick, MD

mean electrical activity for 10 consecutive breaths at each speed was calculated for each horse and expressed in arbitrary units. Data was analyzed using a one-way analysis of variance with speed as the main factor. A significance level of $P < 0.05$ was selected.

Results

Electrodes were placed successfully in all horses. Complications included inadvertent puncture of the external carotid artery during electrode implantation with minor hemorrhage (n=2) and electrode removal by the animal before the data was collected (n=2). When this occurred, the procedure was aborted and repeated two weeks later.

Phasic stylopharyngeus muscle activity increased at the end of expiration and peaked early during inspiration while tonic activity was present during expiration (Figure 6).

The peak EMG activity of the stylopharyngeus muscle increased significantly with exercise intensity (Figure 7). As treadmill speed increased from HR_{max50} to HR_{max75} and from HR_{max75} to HR_{max}, peak inspiratory EMG activity of the stylopharyngeus muscle increased (P=0.001) in each horse: mean value ± SEM were 2.44 ± 0.493 arbitrary units (a.u.) at HR_{max50}, 5.18 ± 0.965 a.u. at HR_{max75} and 8.36 ± 1.321 a.u. at HR_{max} (Figure 8). As speed increased from HR_{max50} to HR_{max75} and from HR_{max75} to HR_{max}, mean electrical activity of the stylopharyngeus muscle increased significantly ($P< 0.001$) in each horse from 0.468 ± 0.0628 a.u., to 1.34 ± 0.294 a.u. and to 2.58 ± 0.471 a.u., respectively (Figure 7). There was no significant effect of treadmill speed on tonic electromyographic activity of the stylopharyngeus muscle, but there was a trend towards an increase in activity (P=0.089). As speed increased from HR_{max50} to HR_{max75} and from HR_{max75} to

HR_{max}, tonic activity increased from 1.38 ± 0.376 a.u. to 1.78 ± 0.414 a.u. and to 1.96 ± 0.42 a.u., respectively.

Discussion

The results of this study demonstrate that the stylopharyngeus muscle has a phasic inspiratory activity that increases with speed and breathing intensity in exercising horses. Therefore, we conclude that the stylopharyngeus muscle functions to support and dilate the dorsal nasopharyngeal wall in exercising horses.

The electromyographic activity of the stylopharyngeus muscle was synchronous with the breathing cycle and tonic activity was present during early expiration. Phasic activity began at the end of expiration and increased to peak activity during early inspiration, similar to the activity measured in goats (Feroah 2000). This pattern is a characteristic feature of upper airway muscles in mammals, in which a very rapid increase in phasic activity precedes the onset of respiratory pump muscle activation (Kuna 1991). Such a sequence of activity is essential to preserve airway stability and patency prior to the onset of negative driving pressures that generate airflow. Minute ventilation increases from 60 liters to in excess of 1700 liters as horses accelerate from a resting state to maximum speed (Erickson *et al.* 1991). Driving pressures of –40 cm H_2O are required to create airflows needed to maintain minute ventilation in horses during intense exercise. Contraction of skeletal muscles is the principal means by which the nasopharynx and larynx achieve dilation and do not collapse in the face of such negative driving pressures.

We observed that the peak and mean electrical activity of the stylopharyngeus muscle increased significantly with speed, likely because breathing effort and exertion increased as treadmill speed increased. More negative driving pressures are required to increase airflow and minute ventilation as the demand for oxygen by the tissues increases. Negative upper airway pressures have been shown to reflexly augment phasic inspiratory and tonic EMG activity in many upper airway dilator muscles and to play an important role in regulating EMG activity of these muscles during breathing (Van Lunteren 1983, Horner 1991, Van der Touw 1994). This reflex is thought to increase airway patency. Pharyngeal size and stability are major factors influencing airway collapsibility. Firstly, the narrower the airway, the more vulnerable it is to closure and the more dependent it is on pharyngeal dilator tone for patency. Secondly, the narrower the airway the greater the inspiratory force necessary to achieve an adequate airflow. The resultant high transmural pressures will favor dynamic pharyngeal collapse during inspiration. Increased contraction of the stylopharyngeus muscle is therefore necessary to enlarge the upper airway as the breathing effort increases to prevent dynamic pharyngeal collapse and upper airway obstruction.

The laryngeal mucosa contains multiple mechanoreceptors that initiate the reflex pathways involved in recruitment of upper airway muscles (Sant'Ambrogio 1983; Tsubone 1998). The stimulus capable of eliciting this reflex is subatmospheric pressure within the laryngeal lumen (Sant'Ambrogio 1983; Van Lunteren 1983; Kuna 1991). In people, upper airway dilator muscles are activated throughout inspiration via continued stimulation of these transducing elements (Akahoshi 2001). These mechanoreceptors

respond to negative pressures and their activity increases in face of airway obstruction, promoting airway patency (Sant'Ambrogio 1983). The internal branch of the superior laryngeal nerve and the recurrent laryngeal nerve provide the afferent pathways for the laryngeal mucosal receptors (Widdicombe *et al.* 1988). Sectioning of the superior laryngeal nerve decreased the EMG activity of the pharyngeal dilator muscles in response to upper airway negative pressure, and decreased the level of negative pressure required to elicit upper airway collapse (Horner 1991). Other pathways are also involved in recruiting upper airway muscles. In goats, the activity of the stylopharyngeus muscle increases with augmented inspiratory drive achieved with hypercapnia and then decreases parallel to a decrease in diaphragmatic muscle activity (Feroah 2000). Locomotion also influences respiratory frequency and airway muscle activity. It is therefore likely that all of these mechanisms are involved in modulating the activity of the stylopharyngeus muscle during exercise in horses.

Desensitization of the laryngeal mechanoreceptors has been shown in horses to induce nasopharyngeal collapse during exercise (Holcombe *et al.* 2001). Therefore, further work is needed to evaluate how the stylopharyngeus muscle responds to these different stimuli and in particular to the laryngeal mecanoreceptors in order to understand what leads to failure of stylopharyngeus recruitment in clinical cases of nasopharyngeal collapse in horses.

APPENDIX

Figure 1: Illustration of the origin and insertion of the stylopharyngeus muscle. Notice how the muscles originate on the axial aspects of the stylohyoid bones and insert on the muscles of the dorsal pharynx.

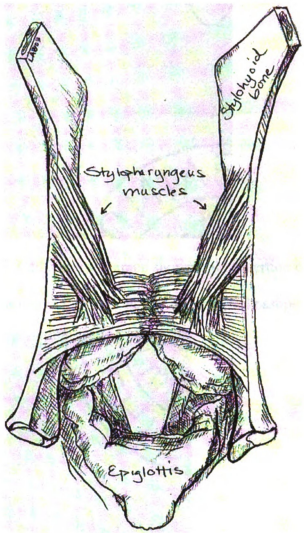


Fig 2a: Endoscopic image of the guttural pouch. Notice the origin of the stylopharyngeus muscle (arrow) on the medial aspect of the stylohyoid bone.

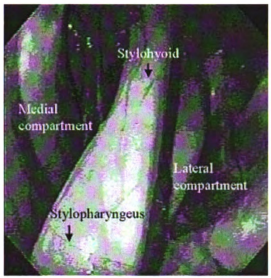


Fig 2b: Cadaveric specimen showing the insertion of the stylopharyngeus muscle (white asterisk) on to the musculature of the dorsal nasopharynx.

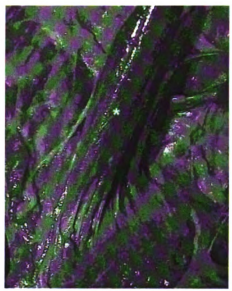


Fig 3: Endoscopic image of the nasopharynx of a horse running on the treadmill following bilateral glossopharyngeal nerve block. Notice that the dorsal wall of the nasopharynx has collapsed, obscuring the view of the corniculate processes of the arytenoids cartilages.

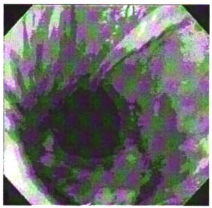


Fig 4: Endoscopic view of the nasopharynx of a horse following right glossopharyngeal nerve block, while the nares are occluded. Notice that only the right hemipharynx has collapsed, while the left side remains in a normal position.

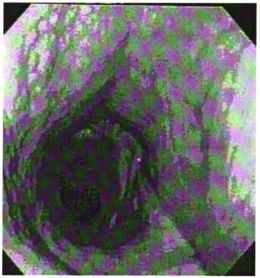


Fig 5: Peak inspiratory pressure in 6 horses during treadmill exercise with and without bilateral glossopharyngeal nerve block. White bars: unblocked trials; Grey bars: with glossopharyngeal nerve anesthesia.

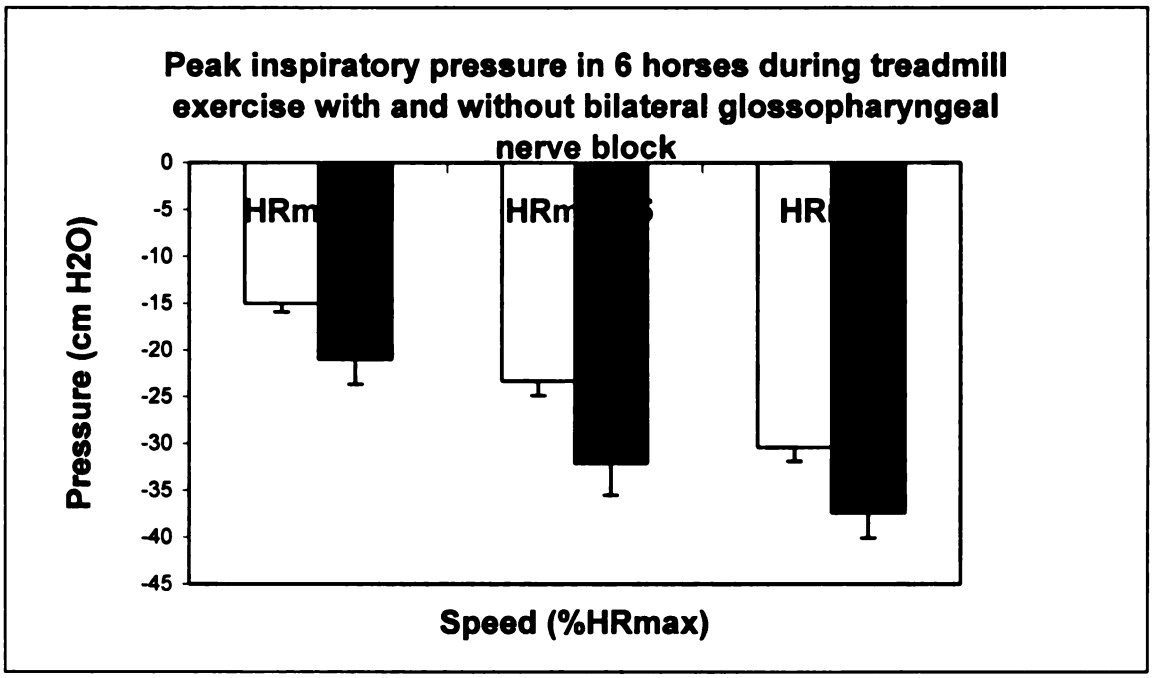


Figure 6. Recording of upper airway pressure (top tracing) and moving time averaged EMG activity of the stylopharyngeus muscle. Notice that the EMG activity of the stylopharyngeus muscle peaks during the early inspiration phase.

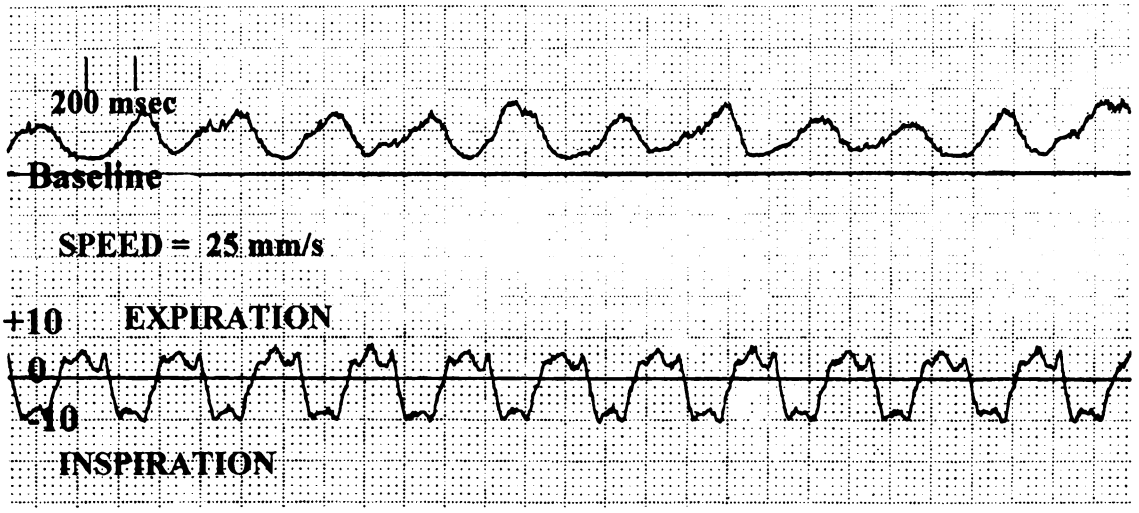


Figure 7. Moving time averaged EMG activity of the stylopharyngeus muscle (top tracing) and raw EMG activity (bottom tracing) recorded at HR_{max50}, $HR_{max\ 75}$, and HR_{max}. Notice that the activity increases at each speed. Speed = 10 mm/sec

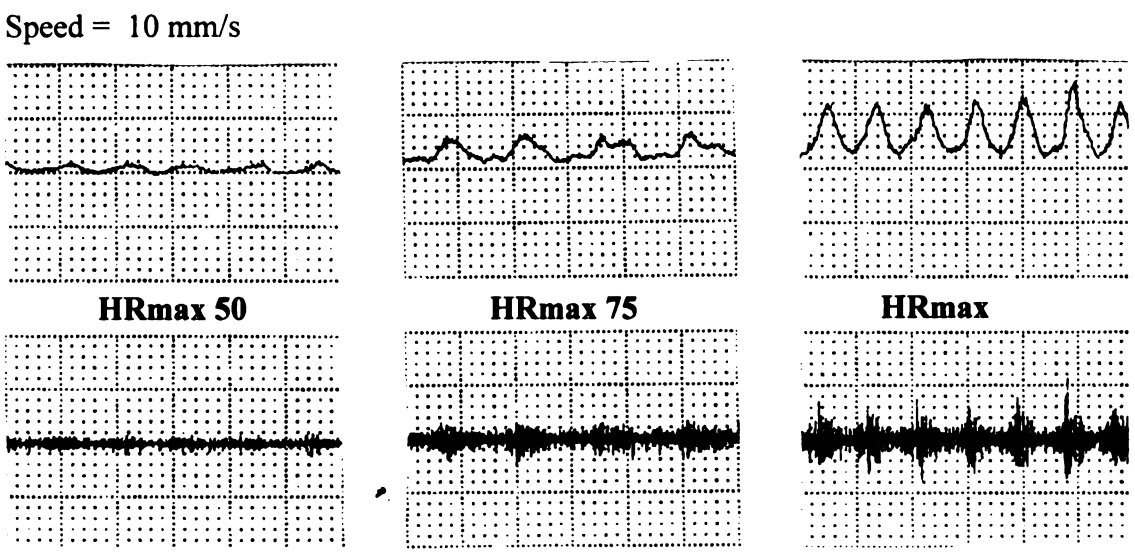
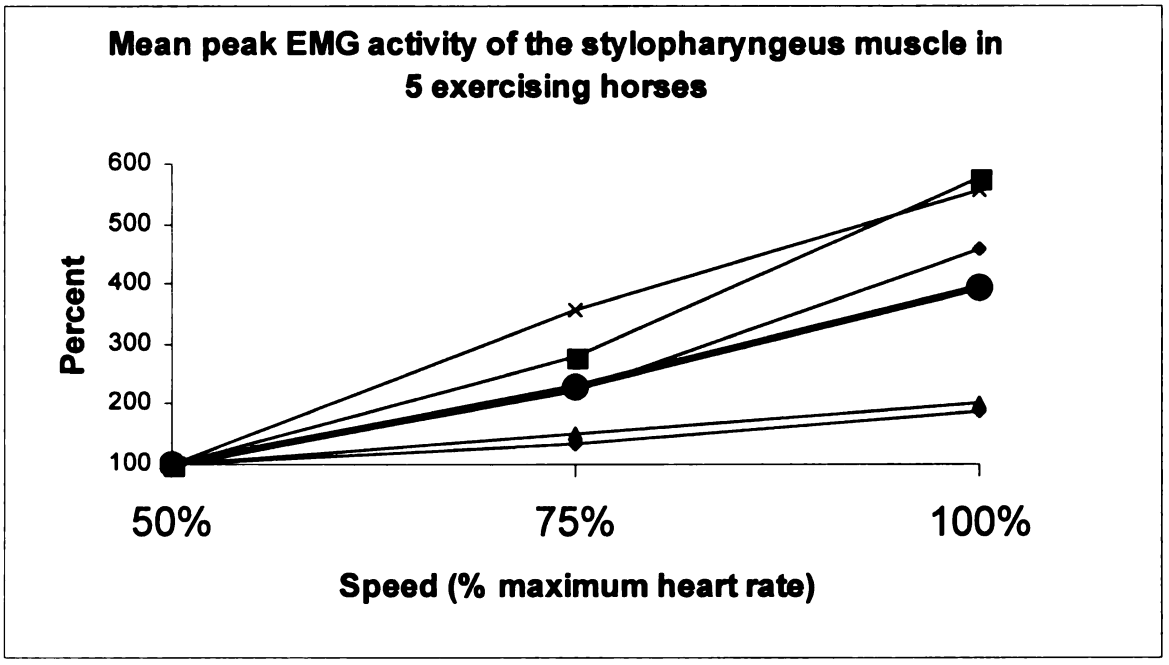


Figure 8. Graph of the peak EMG activity of the stylopharyngeus muscle in 5 exercising horses. Notice that the activity increases at each speed for each horse. The thick black line represents the average of the 5 horses.



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