Role of the hypoglossal nerve in equine nasopharyngeal stability

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Submitted 3 September 2008; accepted in final form 15 May 2009

Cheetham J, Pigott JH, Hermanson JW, Campoy L, Soderholm LV, Thorson LM, Ducharme NG. Role of the hypoglossal nerve in equine nasopharyngeal stability. *J Appl Physiol* 107: 471–477, 2009. First published June 4, 2009; doi:10.1152/japplphysiol.91177.2008.—The equine upper airway is highly adapted to provide the extremely high oxygen demand associated with strenuous aerobic exercise in this species. The tongue musculature, innervated by the hypoglossal nerve, plays an important role in airway stability in humans who also have a highly adapted upper airway to allow speech. The role of the hypoglossal nerve in stabilizing the equine upper airway has not been established. Isolated tongues from eight mature horses were dissected to determine the distal anatomy and branching of the equine hypoglossal nerve. Using this information, a peripheral nerve location technique was used to perform bilateral block of the common trunk of the hypoglossal nerve in 10 horses. Each horse was subjected to two trials with bilateral hypoglossal nerve block and two control trials (unblocked). Upper airway stability at exercise was determined using videoendoscopy and measurement of tracheal and pharyngeal pressure. Three main nerve branches were identified: medial and lateral branches and a discrete branch that innervated the geniohyoid muscle alone. Bilateral hypoglossal block induced nasopharyngeal instability in 10/19 trials, and none of the control trials (0/18) resulted in instability (P < 0.001). Mean treadmill speed (±SD) at the onset of instability was 10.8 ± 2.5 m/s. Following its onset, nasopharyngeal instability persisted until the end of the treadmill test. This instability, induced by hypoglossal nerve block, produced an inspiratory obstruction similar to that seen in a naturally occurring equine disease (dorsal displacement of the soft palate, DDSP) with reduced inspiratory and expiratory pharyngeal pressure and increased expiratory tracheal pressure. These data suggest that stability of the equine upper airway at exercise may be mediated through the hypoglossal nerve. Naturally occurring DDSP in the horse shares a number of anatomical similarities with obstructive sleep apnea. Study of species with extreme respiratory adaptation, such as the horse, may provide insight into respiratory functioning in humans.

dorsal displacement; soft palate; velopharynx; nasopharynx

**AT MAXIMAL EXERTION, THE DOMESTIC HORSE (Equus caballus) has a maximal oxygen uptake (Vo2max) of ~160 ml/kg per min, which is 40 times the value at rest (19, 50). This level of oxygen demand is higher than that predicted by the general relationship with body weight in mammals (30, 48), and the increase is far higher than the six- to eight-fold increase found in endurance-trained human athletes (45). This high oxygen demand has produced extreme adaptation of the equine upper airway, and failure at high levels of exertion most commonly occurs in this zone. This level of specialization makes the horse an interesting model in which to study failure mechanisms of other extremely adapted species such as the human upper airway, which is uniquely adapted for speech. Although all mammals use nasal breathing at rest, in the horse, the caudal border of the soft palate lies ventral to the rostral body of the epiglottis, except during deglutition, resulting in obligate nasal breathing at maximal exertion (12). In ~10–20% of horses, the soft palate becomes displaced dorsal to the epiglottis during maximal exercise (31, 33, 39, 42), and this results in expiratory obstruction associated with soft palate vibration and expiratory noise (18, 25, 36) as seen in snorers (15). Clinically, this condition is referred to as dorsal displacement of the soft palate (DDSP) and produces resistive breathing with impaired athletic performance attributable to a reduction in minute volume, tidal volume, and oxygen consumption (21). The tongue plays an important role in airway stability, and its activity is mediated by the hypoglossal nerve (46). Two subdivisions of the hypoglossal nucleus correspond to peripheral nerve branches, which are anatomically divided and functionally correlated. The dorsal subdivision of the hypoglossal nucleus contributes axons to the lateral branch of the hypoglossal nerve, which innervates the extrinsic retruders (styloglossus and hyoglossus) and their homologous intrinsic muscles (34, 35, 46). The ventral subdivision of the hypoglossal nucleus supplies motor axons to the medial branch, which innervates the extrinsic protruders (genioglossus and geniohyoid muscles) and the horizontal and vertical components of the intrinsic tongue muscles (2, 13, 14). Electrical stimulation of the hypoglossal nerve increases upper airway dilation and patency in rats, rabbits, dogs, and humans (6, 7, 9, 51). The role of the hypoglossal nerve in airway stability in horses has not been established. Previous investigations in horses have focused on the muscles controlling the soft palate and the position of the laryngohyoid apparatus (18, 25). Targeted local anesthesia of the pharyngeal branch of the vagus nerve (X) induces dysfunction of the palatineus and palatopharyngeus muscles and results in DDSP at rest (25). Bilateral resection of the paired thyrohyoid muscles, which attach to the caudal border of the thyrohyoid bones and run to the ipsilateral thyroid lamina, produces DDSP at slow-speed exercise (18). Only one study has evaluated the role of the hypoglossal nerve in airway stability in the horse (26). This study induced hypoglossal dysfunction at the level of the guttural pouch (diverticulum of the external auditory meatus) and produced epiglottic retroversion with inspiratory obstruction (26). As a result, it was not possible to assess the effect on nasopharyngeal stability. Anatomical nomenclature in the horse differs from that in humans. In humans, the airway posterior to the soft palate is described as the velopharynx. In the horse, the same section of airway is termed nasopharynx (Fig. 1).

The precise distribution of the hypoglossal nerve to the equine tongue muscles and the role of these muscles in airway stability have not been determined. Our objective was to test the hypothesis that local blockade of the hypoglossal nerve...
induces upper airway instability in horses. Our findings support this hypothesis. As a prelude to this investigation, it was necessary to more accurately describe the distal anatomy of the equine hypoglossal nerve.

MATERIALS AND METHODS

Gross Dissection

Isolated tongues with attached larynx and hyoid apparatus were collected at necropsy less than an hour after euthanasia from five horses without a history of upper airway disease (mean age 5.8 yr, range 4–8 yr; 3 geldings, 2 mares; 4 thoroughbreds, 1 quarter horse; mean bodyweight 445 kg, range 380–450 kg). A preliminary dissection of the tongue musculature was performed, and the specimens were fixed in 10% formalin. Hypoglossal nerves were subsequently dissected bilaterally and photographed with a high-speed camera (Kodak, Rochester, New York) to catalog branching of the hypoglossal nerve. The distance from the point at which the hypoglossal nerve crossed lateral to the ceratohyoid bone to its division into medial and lateral branches and the dorsoventral and mediolateral dimensions of the hypoglossal nerve 10 mm rostral to the ceratohyoid bone were measured using a micrometer (Craftsman 38668) and recorded.

Hypoglossal Nerve Block

Animals. Ten mature horses (6 thoroughbreds, 1 standardbred, 1 quarter horse, 1 thoroughbred cross, 1 warmblood, 4 females, 6 neutered males, mean age 7.8 yr, range 2–17 yr) with no history of upper airway disease were used. All procedures complied with federal and state regulations and were approved by the Cornell University institutional animal care and use committee. Horses were determined to be in good condition and athletically fit on the basis of a physical examination. Horses without a history of upper airway disease (mean age 5.8 yr, range 4–8 yr; 3 geldings, 2 mares; 4 thoroughbreds, 1 quarter horse; mean bodyweight 445 kg, range 380–450 kg) were shod with flat, aluminum shoes with toe clips. Close-fitting neoprene boots (Professional’s Choice; Professional’s Choice Sports Medicine Products, Spring Valley, CA) were placed on the lower limbs to prevent injuries from interference. A nylon halter was used to decrease the chance of breakage. Horses were fasted for 3 h before any exercise trial (17). At the end of each exercise period, horses were bathed and cooled out appropriately.

Instrumentation. Data recorded during exercise trials included heart rate, tracheal and nasopharyngeal pressure, electrocardiogram monitoring, and accelerometer measurements. Nasopharyngeal and laryngeal movements were recorded using a flexible videendoscope (Olympus GIF-140) that was passed into the nasopharynx via the left ventral nasal meatus and secured. Heart rate was measured by an on-board monitor (Hippocard Systems, Lexington, KY). Data were recorded onto DVD disks for subsequent analysis. Tracheal and nasopharyngeal pressure were measured using two Teflon catheters (1.3 mm ID, NeoFlon; Cole-Parmer, Chicago, IL) placed so the ports lie in the pharynx, level with the opening of the left guttural pouch ostium and in the trachea 30 cm distal to the rima glottidis (17, 37). Positioning of both catheters was confirmed by video endoscopy. The catheters were attached to differential pressure transducers (Ceslos LCVR; Ceslos Transducers Products, Canoga Park, CA) referenced to atmospheric pressure and calibrated from −70 to 70 mmHg (17, 37). The pressure catheters were in phase from 1–20 Hz.

Exercise protocol. At time 0, the treadmill was started and accelerated to 4 m/s. After 1 min at 4 m/s, the treadmill was accelerated to 6 m/s and maintained at that speed for 1 min, and then the speed was increased to 10 m/s and held at that speed for 1 min. Each subsequent minute, the treadmill was accelerated by 1 m/s until the horse was no longer capable of maintaining its position near the front of the treadmill. All horses were run under control conditions to determine that they had a normal upper respiratory tract at exercise. One further control trial and two trials with hypoglossal block were then performed with a randomized block design so that each horse had a total of two control trials and two blocked trials. A minimum of three days was allowed for recovery between each trial.

Hypoglossal Nerve Block

Using data obtained in the anatomic study, bilateral hypoglossal nerve block was performed at the level of the ceratohyoid bones. Hypoglossal nerve block was performed using a peripheral nerve locator (Innervator 232; Fisher & Paykel Healthcare, Auckland, New Zealand) with an initial output of 2 mA, a frequency of 2 Hz, and a pulse duration of 0.15 ms. A disposable ground electrode (Delmar Reynolds Medical, Irvine, CA) was placed cutaneously. A 50-mm stimulating needle (Stimuplex Insulated Needle; Braun Medical, Be-
Hypoglossal Nerve in Equine Nasopharyngeal Stability

Ten hypoglossal nerves and sets of tongue musculature were dissected. In all specimens, the hypoglossal nerve was identified exiting the hypoglossal foramen, coursing rostroventrally along the wall of the medial compartment of the guttural pouch. A small branch was identified, which ran caudally, anastomosed with the first cervical nerve, and then ran rostrally with a significant dorsal deviation to enter the thyrohyoid muscle from its dorsal aspect. The main nerve trunk continued to run rostrally along the ventrolateral aspect of the stylohyoid bone with the lingual artery. A small branch exited, perpendicular to the nerve, through the caudal aspect of the hyoglossus muscle to the paired hyoepiglottic muscles located medially. Rostral to this branch, the nerve ran lateral to the ceratohyoid bone, between the pterygoid and the hyoglossus muscles, at a depth of 2–4 cm from the intermandibular skin surface. At this location, the nerve was lateromedially flattened. At 10 mm rostral to the rostral end of the ceratohyoid bone, it measured (mean ± SE) 1.8 ± 0.2 mm from lateral to medial and 4.3 ± 0.5 mm from dorsal to ventral. The nerve then diverged from the path of the lingual artery at the caudal aspect of the hyoglossus muscle and remained lateral to the hyoglossus muscle.

Rostral (48.3 ± 15.0 mm) to the ceratohyoid bone between the hyoglossus and styloglossus muscles, the main hypoglossal nerve trunk divided into a number of branches (Fig. 2). This pattern of division of the main trunk was consistent across all specimens studied. Division occurred between the mandibular salivary duct laterally and the lingual vein medially. A small branch ran rostroventrally to innervate the geniohyoid muscle along its dorsal margin (Fig. 2A). The remaining nerve divided into two main branches, medial and lateral. The lateral branch (Fig. 2C) immediately divided into multiple smaller branches and innervated the hyoglossus and styloglossus muscles. The medial branch (Fig. 2B) continued rostrally, wrapping rostro- dorsally around the hyoglossus muscle, then sending multiple small secondary nerve branches to innervate the genioglossus muscle (Fig. 3). Twelve to twenty-two secondary branches were identified for each genioglossus muscle. No clear division was identified between the horizontal, vertical, and oblique neuromuscular compartments of the genioglossus.

Hypoglossal Block

The criteria for injection, namely negative blood aspiration, cessation of twitch response after test injection of mepivicaine, and absence of resistance to injection, were met in all blocks. The threshold current for injection was 0.2–0.4 mA. Tongue tone was reduced, on the basis of manual extension of the tongue, following two blocks.

Two horses only underwent one control trial because of a low-grade lameness that developed during exercise testing. One of these horses only had one trial with hypoglossal nerve block for the same reason. DDSP or other signs of nasopharyngeal instability did not occur during any of the control trials (0/18 control trials); 10 trials with hypoglossal block produced DDSP (10/19), and this difference was significant ($P < 0.001$, results).

RESULTS

Gross Dissection

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Fischer’s Exact Test). Two trials with hypoglossal block also resulted in epiglottic retroversion. This did not occur in any of the control trials.

There was some variability between horses. Three horses did not show DDSP in any of the blocked trials. The remaining seven horses all showed DDSP in one or both trials (Fig. 4). To control for this variability between horses, a logistic regression model was fitted to the data with horse identity, repetition (1 or 2), and the presence or absence of hypoglossal block as predictor variables. There was a significant effect of horse identity in this model ($P = 0.03$), and horses were more likely to displace in the presence of hypoglossal block ($P < 0.0001$). There was no significant effect of repetition number ($P = 0.41$), and overall model fit was good ($r^2 = 0.82$).

**Airway Pressure Profile**

Mean speed (± SD) at the onset of DDSP was 10.8 ± 2.5 m/s. Following its onset, DDSP persisted until the end of the treadmill test. Dorsal displacement of the soft palate, induced by hypoglossal nerve block, reduced inspiratory and expiratory pharyngeal pressure and increased expiratory tracheal pressure (Fig. 5). All four measures of airway pressure increased with speed in control trials. The overall model fits for three measures of airway pressure were good with adjusted $r^2$ values of 0.79 for expiratory pharyngeal pressure, 0.72 for inspiratory pharyngeal pressure, and 0.78 for inspiratory tracheal pressure. Model fit for expiratory tracheal pressure was moderately good ($r^2 = 0.47$).

**DISCUSSION**

**Summary**

We provide the first experimental evidence that stability of the equine nasopharynx (analogous to the human velopharynx) at exercise is mediated through the hypoglossal nerve. We demonstrate that blockade of the hypoglossal nerve at the level of the ceratohyoid bone can produce dorsal displacement of the soft palate in horses. This study evaluated the effect of hypoglossal block on nasopharyngeal stability; it did not distinguish between the effects of the intrinsic and extrinsic tongue musculature.

**Novel Observations**

Equine nasopharyngeal instability, manifested as DDSP, occurred at high levels of exertion (mean treadmill speed, 11 m/s) following hypoglossal nerve block. The absence of DDSP at rest and lower speeds may suggest that the hypoglossal nerve may only be important in equine nasopharyngeal stability at high levels of aerobic exercise. This finding is in contrast to the instability produced by blockade of the pharyngeal branch of the vagus nerve, which produces DDSP at rest (25).

The main limitation of the study is the difficulty in determining the efficacy of hypoglossal nerve block. Tongue tone was manually assessed in all cases before treadmill testing but was only found to be reduced in two of the trials, which subsequently exhibited DDSP. Epiglottic retroversion occurred in two trials with hypoglossal block attributable to induced dysfunction of the paired hyoepiglotticus muscles. This muscle is important for maintaining airway patency in other species (3, 4), and dysfunction produces an inspiratory obstruction in horses (26). It is possible that this may have influenced the occurrence of DDSP in these two trials.

**Methodological Issues**

The threshold current for injection ranged from 0.2 mA to 0.4 mA. Some authors indicate that a minimal amplitude of 0.2 mA might indicate intraneural stimulation and might risk intrafasicular injection and iatrogenic nerve damage (28). We did not experience any complications associated with this technique. This is consistent with reports that a minimal amplitude of <0.2 mA can be used safely (24, 27). In humans, the nerve location technique is safe with an incidence of peripheral neuropathy of ~2.3/10,000 regional anesthesia procedures (5).

Some variability was identified between horses in terms of the effect of bilateral hypoglossal nerve block. There are two possible explanations for this. Some authors suggest that immediate cessation of the motor twitch following test injection
(Raj test) suggests proximity to the nerve (8); however, in the absence of intraneural injection, instantaneous sodium channel blockade cannot be the cause of immediate cessation of the motor response. Rather, this phenomenon is more likely to be the result of physical displacement of the tissues, along with the nerve, away from the needle. As such, the phenomenon may be observed at any distance from the nerve (49). It is therefore possible to see an immediate loss of motor response when injecting while still a significant distance from the nerve. This may have limited the accuracy of the needle placement and hypoglossal nerve block in this study and may have produced some of the variability between horses.

Alternately, horses with naturally occurring DDSP at exercise have recently been shown to have a more anteriorly (ventrally) positioned basihyoid bone at rest (10). It is possible that there is a continuum of susceptibility to DDSP on the basis of hyoid positioning and that horses at one end of this continuum are less likely to manifest DDSP following hypoglossal nerve block.

**Physiological Significance**

The airway pressure profile of DDSP, induced by hypoglossal nerve block, is similar to that found in horses with naturally occurring DDSP (18, 42, 44). The condition produces an expiratory obstruction (increased expiratory tracheal pressure) and diversion of airflow through the mouth (reduced inspiratory and expiratory pharyngeal pressures).

This study demonstrates that blockade of the hypoglossal nerve induces nasopharyngeal instability at exercise. It does not distinguish between the effects of individual tongue muscles. We propose that hypoglossal nerve block may induce nasopharyngeal instability in horses either by allowing caudal retraction of the hyoid apparatus or by preventing protrusion of the genioglossus muscle.

The extreme adaptation of the equine upper airway to high levels of sustained aerobic exercise may provide a useful model for the study of the human upper airway, which is also extremely adapted to facilitate speech. During breathing in humans, pharyngeal patency is preserved by activation of the genioglossus (29). The primary stimulus to the genioglossus muscle is negative airway pressure (1, 32). These reflexes have not been described in the horse. Pharyngeal obstruction is more likely in humans with a more inferiorly (ventrally) positioned larynx (38, 43), and genioglossus muscle responsiveness to partial oropharyngeal obstruction is also greater in men with a hyoid bone positioned more inferiorly (38). It has recently been shown that a more posteriorly (dorsally) positioned basihyoid bone is associated with improved performance following surgery for DDSP in horses (11) and that horses with DDSP at exercise have a more anterior (ventral) position of the basihyoid bone at rest (10). These findings that a more posterior (dorsal) position is associated with improved performance are consistent with the situation in humans in which a more anteriorly (ventrally) positioned basihyoid bone is associated with decreased airway stability in patients with obstructive sleep apnea (OSA) (43, 47). In addition, caudal retraction of the hyoid apparatus during sleep in man can lead to pharyngeal collapse (20, 22). This is similar to the sequence of caudal retraction, swallowing, caudal retraction, and then DDSP seen in the horse (16).

DDSP occurs naturally at high-speed exercise and produces a predominantly expiratory obstruction. In contrast, OSA occurs during sleep and is primarily inspiratory in nature. Despite these differences, this study suggests that there are some similarities between DDSP in horses and OSA in humans. We propose that the anatomy of the equine nasopharynx, with the epiglottis dorsal and locked into the caudal border of the soft...
palate, may predispose horses to expiratory rather than inspiratory obstruction in the presence of nasopharyngeal instability.

**REFERENCES**


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