Effect of upper airway negative pressure on proprioceptive afferents from the tongue

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Brancatisano, A., P. Davis, T. Van der Touw, and J. R. Wheatley. Effect of upper airway negative pressure on proprioceptive afferents from the tongue. J. Appl. Physiol. 86(4): 1396–1401, 1999.—We examined whether receptors in the tongue muscle respond to negative upper airway pressure (NUAP). In six cats, one hypoglossal nerve was cut and its distal end was prepared for single-fiber recording. Twelve afferent fibers were selected for study on the basis of their sensitivity to passive stretch (PS) of the tongue. Fiber discharge frequency was measured during PS of the tongue and after the rapid onset of constant NUAP. During PS of 1–3 cm, firing frequency increased from 17 ± 7 to 40 ± 11 (SE) Hz (P < 0.01). In addition, 8 of the 12 fibers responded to NUAP (10–30 cm H2O), with firing frequency increasing from 23 ± 9 to 41 ± 9 Hz (P < 0.001). In two fibers tested, the increase in firing frequency in response to NUAP was not altered by topical anesthesia (10% lignocaine) applied liberally to the entire upper airway mucosa. Our results demonstrate that afferent discharges from the hypoglossal nerve are elicited by 1) stretching of the tongue and 2) NUAP before and after upper airway anesthesia. We speculate that activation of proprioceptive mechanoreceptors in the cat’s tongue provides an additional pathway for the reflex activation of upper airway dilator muscles in response to NUAP, independent of superficially located mucosal mechanoreceptors.

upper airway control; hypoglossal nerve; muscle spindles

Previous studies have demonstrated that negative pressure applied to the upper airway of animals and humans causes reflex activation of a number of upper airway dilator muscles, including the alae nasi (22), genioglossus (10, 15, 16, 22), cricothyroid (14), posterior cricoarytenoid (14, 22), sternohyoid and sternothyroid (14), and soft palate muscles (21). It has been proposed that upper airway muscle recruitment by negative pressure protects the upper airway against collapse from negative intraluminal pressures (14, 22). Although the precise afferent pathways for these reflexes have not been determined, pressure-sensitive mechanoreceptors in the upper airway have been demonstrated to respond to negative airway pressure (11, 12, 15, 16, 19). Furthermore, many of these receptors are superficial (located in the mucosal and submucosal tissues), because they are affected by local anesthesia (9, 14). However, a role for more deeply located pressure receptors (i.e., within muscle tissue) is not precluded. Negative upper airway pressure (NUAP) may activate muscle mechanoreceptors by stretching and deforming muscle tissue. Hence, an alternate group of nonsuperficial upper airway receptors could participate in the detection of NUAP. In addition, these muscle mechanoreceptors may be important in the reflex response of individual upper airway muscles to NUAP. This mechanism would permit upper airway muscles to respond differentially to the same negative pressure, with the stimulated activity partially dependent on the level of local mechanoreceptor activation. However, there is no information concerning the afferent nerve traffic arising from such mechanoreceptors in upper airway muscles when they are stimulated by NUAP.

By recording from the hypoglossal nerve (motorsensory nerve to the tongue muscles), we examined the role of NUAP in stimulation of muscle receptors from the tongue. The tongue muscle was selected for study on the basis of its recognized importance in the control and maintenance of upper airway patency, because of the relative accessibility of the hypoglossal nerve, and because the hypoglossal nerve does not carry sensory information from mucosal surfaces in the upper airway.

METHODS

Studies were performed on six anesthetized, paralyzed (gallamine triethiodide, 4 mg/kg iv) and mechanically ventilated supine adult cats [weight 3.0 ± 0.8 (±SD) kg]. After animals were premedicated with acetylpromazine (0.2 mg/kg im), general anesthesia was induced by placing the animal in a box filled with a mixture of halothane and 100% oxygen delivered by a ventilatory pump. After anesthesia was induced, the halothane circuit was switched to a face mask so that anesthesia could be maintained while femoral venous and arterial catheters were inserted for the systemic administration of saline, anesthetics, and paralyzing agents and for the measurement of arterial blood pressure. Thiopentone sodium (10–20 mg/kg iv) was then injected to allow insertion of an endotracheal tube, which was used for the subsequent continued administration of halothane. Rectal temperature was monitored and maintained at 37.5–38.5°C by using an infrared lamp. End-tidal CO2 was measured with an infrared gas analyzer (Morgan 901) and was maintained at 4–5% by adjusting ventilation. Arterial blood pressure (±200 cmH2O pressure transducer; Celesco) and heart rate were continuously monitored as indexes of the depth of anesthesia. The investigation was approved by the University of Sydney Animal Care and Ethics Committee.

A tracheostomy was performed, and the caudal tracheal end was connected to a halothane anesthesia circuit and ventilator, while the laryngeal end was connected to a pressure transducer (± 50 cmH2O; Celesco) to record upper airway pressure (Fig. 1). The esophagus was ligated, and the upper airway was sealed by placing a specially constructed plastic mask (made airtight with petroleum jelly) over the cat’s nose.
Negative airway pressure was applied to upper airway via a perforated tube placed in the mouth. Tongue was stretched by using a displacement transducer.

Hypoglossal nerve isolation. The hypoglossal nerve was dissected free from where it crossed the carotid artery, and it was sectioned just caudal to where it branches to the tongue muscles. The distal end of the medial division of the hypoglossal nerve was placed on a pair of platinum electrodes and was sutured to the tip of the tongue. The distal end of the nerve was then connected to a displacement transducer (model 305B, Cambridge Technology, Watertown, MA) to permit the measurement of tongue stretching.

Experimental protocol. Single fibers were selected for study on the basis of a positive ENG response to passive stretch (PS) of the tongue. Seven of the 12 fibers were tonically active (Fig. 2) and the remaining five were silent in the absence of stimulation (Fig. 3). In response to a constant stretch of the tongue, there was an initial burst of activity which then decreased to a steady rate of discharge above the control level (Fig. 2). The initial burst of activity occurred more frequently at the larger displacements. During steady-state PS of between 1 and 3 cm, firing frequency increased in the group as a whole from 17 ± 7 to 40 ± 11 Hz (Fig. 4; P < 0.01). Affenter activity was transiently inhibited after the removal of PS but then returned to control levels (Fig. 2).

Step changes in negative pressure applied to the upper airway led to an immediate increase in ENG activity (Fig. 3). In addition, at the higher NUAPs, there was an initial burst of activity which then decreased to a steady rate of discharge above the control value for the duration of the negative pressure. Of the 12 fibers that responded to PS, 8 responded to NUAP between −10 and −30 cmH₂O, with firing frequency increasing from 23 ± 9 to 41 ± 9 Hz (Fig. 4; P < 0.001). For two fibers tested at multiple pressure levels and degrees of tongue displacement in the same cat, there was a positive linear relationship between the increase in frequency of fiber discharge (above control) and both the degree of tongue stretch (Fig. 5), and the level of NUAP (Fig. 6).

In the two fibers tested after local anesthesia was administered, the increase in firing frequency in re-
response to −14 cmH₂O NUAP was not altered by topical anesthesia of the upper airway mucosa. In three to five trials of NUAP, the increase in firing frequency of these two fibers did not change: from 24.6 ± 0 and 13.0 ± 0.5 Hz before local anesthesia, to 20.7 ± 3.0 and 15.4 ± 0.9 Hz after local anesthesia, respectively (Fig. 7).

DISCUSSION

The principal findings of this study are that in anesthetized, supine, tracheostomized cats afferent discharges from the hypoglossal nerve are elicited by 1) mechanical displacement of the tongue and 2) NUAP. In addition, topical upper airway anesthesia does not abolish the afferent discharges elicited by NUAP.

A number of upper airway muscles in both animals and humans are recruited in response to NUAP within the upper airway lumen (10, 16, 22). The genioglossus and hypoglossal nerve afferent activity have been extensively studied, and negative pressure recruitment of genioglossus muscle activity is predominantly mediated via stimulation of pressure-sensitive upper airway mucosal mechanoreceptors (11, 12, 15, 16). This is supported by studies in which topical anesthesia of the upper airway mucosa can abolish or diminish the genioglossus response to NUAP in both animals and humans (9, 15, 16). However, in animals, sectioning of the internal branches of the superior laryngeal nerve markedly reduced but did not eliminate the response to NUAP. This suggests that alternate afferent pathways are involved in this reflex response. The glossopharyngeal and trigeminal nerves have been suggested as possible afferent pathways for reflex recruitment of upper airway muscles in response to NUAP (9, 11, 12, 14), because the nerves are involved in detecting mucosal sensation in the nose, nasopharynx, and oropharynx. In addition to mucosal mechanoreceptors, there are several other possible upper airway receptors. These include slowly and rapidly adapting receptors of the upper airway, proprioceptive nerve endings of the tongue, laryngeal muscle and joint receptors, and other muscle mechanoreceptors.

Critique of method. There were a number of limitations to the methodology employed in the current study. First, passive distension of a fiber and characteristics of the receptive field of the fibers could not be tested. Second, the degree of movement associated with application of the face mask and negative pressure was such that it was not always possible to keep recording from a single fiber by using a teased fiber preparation. Consequently, data sets from individual fibers were frequently incomplete. Third, the experimental setup was unable to measure the degree of tongue displacement induced by negative pressure. Fourth, we were unable to determine whether the afferent activity from the

Fig. 2. Example of afferent activity of a single hypoglossal nerve fiber recorded in response to a step increase in tongue displacement. Top: raw electroneurographic (ENG) activity from the fiber. Middle: instantaneous firing frequency of the fiber. Bottom: tongue displacement. Note tonic discharge of single fiber before stimulus. In response to a constant displacement of the tongue, there was an initial burst of activity which then decreased to a steady rate of discharge above the control level. After reversal of the displacement, afferent activity was briefly inhibited but then returned to control levels.

Fig. 3. Example of afferent activity of a single fiber recorded during a step change in negative upper airway pressure (NUAP). Top: raw ENG activity from the fiber. Middle: instantaneous firing frequency of the fiber. Bottom: NUAP. Note that this fiber was silent before negative pressure. In response to NUAP, there was an initial burst of activity which then decreased to a steady rate of discharge above the control value for the duration of the negative pressure. In addition, note that gradual change in negative pressure after removal of the pressure source was associated with a progressive decrease in firing frequency.
The hypoglossal nerve in mammals and primates is motorsensory to the extrinsic and intrinsic muscles of the tongue and the geniohyoid muscle (8, 17). The extracranial hypoglossal nerve has a larger medial and a smaller lateral division. The medial division gives an intermediate branch to the geniohyoid muscle, then splits into numerous twigs to the genioglossus and other intrinsic muscles of the tongue. The lateral division supplies the styloglossus and hyoglossus. The hypoglossal nerve does not carry any sensory information from the upper airway mucosal surfaces. Thus it is clear from anatomic considerations that the afferent discharges elicited from the hypoglossal nerve in our study did not arise from mucosal or submucosal mechanoreceptors and must have arisen from receptors within the tongue muscle. Moreover, in the fibers we studied, topical upper airway anesthesia did not significantly alter the increase in afferent discharge elicited by NUAP; this supports our hypothesis that the afferent discharges from the hypoglossal nerve did not arise from mechanoreceptors located in the mucosa or submucosa.

The precise nature of the tongue muscle mechanoreceptors stimulated by NUAP in this study remains speculative. Early histological studies failed to show the existence of neuromuscular spindles within the intrinsic and extrinsic musculature of the cat’s tongue (1, 3, 5). Despite this, Cooper (6) recorded activity from single-fiber afferents of the cat hypoglossal nerve in response to stretching of the tongue. More recent studies, using sophisticated histological techniques, have now identified muscle spindles in the tongue muscles of subprimate animals (13, 18, 20). However, the prevalence of muscle spindles in the tongue muscles varies between species. In contrast to subprimate species, muscle spindles are relatively numerous in the intrinsic and extrinsic muscles of the monkey tongue and the human tongue (2, 5, 7).

In the six cats we studied, only 12 afferent fibers were selected on the basis of their sensitivity to PS of the tongue. The paucity of afferent fibers detected is consistent with previous studies that have directly identified muscle spindles in rat tongues. Smith (20) identified between two and seven spindles in four rats studied, whereas O’Reilly and Fitzgerald (18) identified a total

![Fig. 4. Mean firing frequency of 12 single fibers before (control) and during tongue displacement (1–3 cm) (left), and mean firing frequency of 8 single fibers before (control) and during NUAP of −10 to −30 cmH₂O (right). Values of firing frequency were obtained during steady-state fiber discharge. Bars, SE. *P < 0.01. Note increase in afferent discharge in response to both displacement of the tongue and NUAP.](image)

![Fig. 5. Plots of %increase in firing frequency (above control) as function of tongue displacement for 2 fibers. Each point represents a single value. Two displacement runs were performed. Linear regression lines and correlation coefficients (R) are shown. Note that in both fibers there was a positive linear relationship between the increase in frequency of fiber discharge and the degree of tongue displacement.](image)
of eight spindles in eight rat tongues. This contrasts with the numerous muscle spindles identified in primate and human tongues (2, 5, 7). Our results together with numerous reports identifying muscle spindles in primate and subprimate mammals suggest that muscle spindles are the proprioceptive nerve endings responsible for the hypoglossal sensory discharge in the present study.

Several authors have recorded sensory discharges from the medial end branch of the hypoglossal nerve of the cat (1, 6, 23) and monkey (2). Our results are consistent with those of Zapata and Torrealba (23), who provided a detailed and quantitative report of sensory discharges recorded from fine filaments of the hypoglossal nerve in response to PS of the cat's tongue. In both studies, the fibers responded with slow adaptation to mechanical stimulation produced by passive tongue displacements. In addition, most hypoglossal sensory units showed a tonic rate of discharge during resting conditions, whereas some fibers were silent at rest (Figs. 2 and 3). Our study has extended these findings by demonstrating that most hypoglossal sensory fibers that respond to passive displacement also respond to NUAP.

In the present study, we recorded from a number of hypoglossal nerve afferents and found that the firing frequency increased in response to NUAP (Fig. 6). As the upper airway pressure became more negative, fibers showed an accelerated discharge at the onset of the stimulus and a pause at the offset of the stimulus (Fig. 2). Furthermore, we demonstrated that firing frequency was directly and linearly related to the degree of NUAP stimulus (Fig. 6). These observations suggest that the rate of sensory discharge signals not only the intensity of NUAP but also the time course of application and removal of the stimulus. In the fibers we studied, we found a positive linear relationship between the firing frequency and both the amount of tongue displacement (Fig. 5) and the degree of NUAP (Fig. 6). Indeed, the similarity in response patterns between passive tongue displacement and NUAP (in the same fiber) suggests that the mechanism by which negative pressure stimulates proprioceptive afferents is via stretching or deforming the tongue. Recently, Brennink et al. (4) embedded sonomicrometers and electrodes in genioglossus muscle fibers and found that NUAP lengthened the genioglossus muscle. This supports our hypothesis that NUAP in our study stretched or deformed the tongue muscle, hence activating proprioceptive afferents in the same manner as would tongue displacement.

It remains unclear whether afferent activity in the hypoglossal nerve elicited by tongue stretch (or negative pressure) will cause reflex activation of the genioglossus muscle. There are no data in the literature to directly support this assumption. However, the responses demonstrated in our study do provide an additional afferent mechanism that may possibly lead to reflex activation of the tongue muscle in response to negative pressure.

In conclusion, this is the first study to demonstrate that NUAP can stimulate mechanoreceptors in the tongue muscles and that these mechanoreceptors are not superficially located in the tongue mucosal surface. Stretching of the tongue is the most likely mechanism by which negative pressure activates hypoglossal afferent fibers. We speculate that activation of muscle mechanoreceptors, such as tongue muscle spindles, may provide an alternative mechanism for the reflex activation of upper airway dilator muscles in response to NUAP and that this mechanism operates indepen-
dent of superficially located (mucosal) pressure mecha-

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