Soft palate muscle responses to negative upper airway pressure

T. C. Amis, N. O’Neill, J. R. Wheatley, T. Van der Touw, E. Di Somma, and A. Brancatisano. Soft palate muscle responses to negative upper airway pressure. J. Appl. Physiol. 86(2): 523–530, 1999.—The afferent pathways and upper airway receptor locations involved in negative upper airway pressure (NUAP) augmentation of soft palate muscle activity have not been defined. We studied the electromyographic (EMG) response to NUAP for the palatini, tensor veli palatini, and levator veli palatini muscles in 11 adult, supine, tracheostomized, anesthetized dogs. NUAP was applied to the nasal or laryngeal end of the isolated upper airway in six dogs and to four to six serial upper airway sites from the nasal cavity to the subglottis in five dogs. When NUAP was applied at the larynx, peak inspiratory EMG activity for the palatini and tensor increased significantly (P < 0.05) and plateaued at a NUAP of ~10 cmH2O. Laryngeal NUAP failed to increase levator activity consistently. Nasal NUAP did not increase EMG activity for any muscle. Consistent NUAP reflex recruitment of soft palate muscle activity only occurred when the larynx was exposed to the stimulus and, furthermore, was abolished by bilateral section of the internal branches of the superior laryngeal nerves. We conclude that soft palate muscle activity may be selectively modulated by afferent activity originating in the laryngeal and hypopharyngeal airway.

Negative pressure recruitment of upper airway electromyographic muscle (EMG) activity is mediated via a reflex response originating from upper airway mechanoreceptors (8, 9, 15, 27) and having afferent pathways thought to involve the trigeminal, glossopharyngeal, and superior laryngeal nerves. Thus, for muscles such as the genioglossus, receptors located in the nasal, pharyngeal, and laryngeal segments of the upper airway are reportedly involved (2, 8, 9, 15) in the response. The implication from such a distribution of receptor sites is that exposure of any single upper airway segment to NUAP will result in the global recruitment of upper airway dilator muscle activity. However, the soft palate contains both elevator and depressor muscles. Consequently, the resultant effect (on upper airway geometry) of global recruitment of palatal muscles is likely to depend on interactions between antagonists, which, in turn, will depend on relative mechanical advantage and the responsiveness of individual muscles to NUAP.

The afferent pathways for augmentation of soft palate muscle activity with NUAP have not been defined (25). Consequently, the relative contribution (to soft palate muscle recruitment) of receptors located in different topographical zones of the upper airway is not understood. Furthermore, in a previous study in dogs (22), recruitment of individual soft palate muscles by NUAP varied, with recruitment of levator being the least reproducible of the muscles studied. This finding has led us to hypothesize that there are differences in the linkage of individual soft palate muscles to the NUAP reflex (i.e., muscle-specific reflex responses). In addition, the magnitude of individual soft palate muscle responses to differing levels of NUAP (i.e., stimulus-response characteristics) have not been defined. The strength of the response of individual soft palate muscles to NUAP is relevant to their ability to contribute to the resolution and prevention of obstructive apneas in OSA.

Thus the aims of the present study were as follows: 1) localize the negative-pressure-sensitive zones within the upper airway that participate in the NUAP-mediated reflex recruitment of individual palatal muscles and 2) define stimulus-response characteristics for recruitment of individual palatal muscles with NUAP.

METHODS

We studied 11 adult crossbred dogs (5 males, 6 females) under general anaesthesia induced by intravenous pentobarbital sodium (25–30 mg/kg) followed by intravenous α-chloralose (initial dose 25 mg/kg). Anesthesia was maintained with...
an intermittent infusion of a 0.5% α-chloralose solution administered via a cannula inserted into a femoral vein. α-Chloralose produces stable, long-lasting, light anesthesia and has been used widely in animal studies examining upper airway reflexes (11, 23, 26). Anesthesia levels were adjusted, as necessary, to maintain a stable heart rate and blood pressure throughout the study. Seven of the animals were also part of a previous study in which responses of the genioglossus and hyoepiglotticus muscles to NUAP were examined (1). The investigation was approved by the Western Sydney Area Health Service Animal Care and Ethics Committee.

Experimental Preparation

The animals were studied supine with the mouth closed and secured with tape. A tight-fitting face mask was sealed to the animal’s muzzle. Pressure in the mask was measured with a pressure transducer (±100 cmH\textsubscript{2}O, Celesco Transducer Products, IDN Instruments, Dandenong, Victoria, Australia).

The trachea was divided between the fourth and fifth cartilage rings caudal to the cricoid cartilage. Care was taken to preserve the recurrent laryngeal nerves. Separate tracheal cannulas were inserted and fastened to the cranial and caudal cut ends of the trachea. Airflow was measured at the caudal tracheal cannula with a pneumotachograph (Fleish no. 1) coupled to a differential pressure transducer (MP 45, ±5 cmH\textsubscript{2}O, Validyne, Northridge, CA). Tidal volume (\textit{Vt}) was measured by electrical integration of the flow signal. In six dogs (group 1), pressure in the laryngeal lumen was measured with a pressure transducer attached to a side port on the cranial tracheal cannula. With the use of three-way taps, a high-impedance negative-pressure source was connected to either the cranial tracheal cannula or the face mask, thus allowing a constant negative pressure to be applied to the nasal or laryngeal end of the isolated and sealed upper airway.

The remaining five animals (group 2) were prepared in an identical manner except that a cuffed endotracheal tube (internal diameter 5.0 mm, external diameter 6.9 mm, length 23 cm, Mallinckrodt) was passed into the nasopharyngeal airway via the cranial tracheal cannula. The tip of this tube was placed at the nasal choanae, and the cuff was inflated. Negative pressure was then applied to the airway via the endotracheal tube with the applied pressure measured at the caudal end of the tube via a pressure transducer (MP 45, ±100 cmH\textsubscript{2}O, Validyne). The cuff was then deflated, and the tip of the tube was moved caudally by 2–4 cm. The cuff was then inflated, and negative pressure was reapplied. This procedure was repeated until the tip of the tube was caudal to the glottis and in the cranial trachea. In this manner, systematically longer segments of the nasopharyngeal airway were exposed to the NUAP stimulus. The position of the tip of the tube was assessed from a distance scale marked on the tube at the commencement of the study when, with the mouth open, the position of the tube could be related visually to the tube at the commencement of the study when, with the mouth open, the position of the tube could be related visually to the position of the tube at the commencement of the study when, with the mouth open, the position of the tube could be related visually to the position of the tube at the commencement of the study when, with the mouth open, the position of the tube could be related visually to the position of the tube at the commencement of the study when, with the mouth open, the position of the tube could be related visually to the position of the tube at the commencement of the study when, with the mouth open, the position of the tube could be related visually to the position of the tube at the commencement of the study when, with the mouth open, the position of the tube could 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activity with NUAP of between −20 and −40 cmH₂O. Distance along the upper airway was expressed as a percentage of the total distance from the nasal choanae (0%) to the glottis (100%). Group mean data were then analyzed for three zones in the nasopharyngeal airway: 1) measurements obtained at all sites <70% of the distance from the nasal choanae to the glottis, i.e., rostral zone; 2) measurements obtained between the 70% distance point and the glottis, i.e., epiglottic zone; and 3) measurements obtained with NUAP applied below the glottis, i.e., subglottic zone.

Group 1 and group 2 dogs showed similar responses; hence the data, where applicable, have been pooled for analysis. Similar findings were also obtained for both single-step and progressive graded changes for the same level of NUAP (P > 0.05); consequently, these data have also been pooled for analysis. Statistical comparisons were made by using a paired t-test for single-condition paired data and ANOVA (with appropriate adjustment where necessary for unbalanced data) for multiple-condition comparisons. Fisher’s protected least significant difference test was used as a post hoc multiple-comparison technique. P < 0.05 was taken as significant.

**RESULTS**

**Transmission of NUAP Through the Upper Airway**

In all group 1 dogs, the changes in NUAP applied at either the larynx/cranial trachea or the face mask were not transmitted to the opposite end of the upper airway for any NUAP level studied (Fig. 1). In the group 2 dogs, NUAP was transmitted to the face mask in all cases where the tip of the endotracheal tube was at the nasal choanae. Transmission also occurred to varying degrees as the tube was moved caudally. When the tube was in the cranial trachea, partial transmission of the pressure stimulus (~5–50%) to the face mask occurred in three dogs.

**Laryngeal NUAP Application: SLNin Intact**

Palatine. Data for palatine MTA EMG activity were obtained in 10 dogs. During control conditions, respiratory-related phasic inspiratory palatine MTA EMG activity was detected in six dogs, phasic expir-
tory activity was found in four dogs, and low-level tonic activity was present in three dogs. Application of laryngeal NUAP increased palatinus peak inspiratory EMG (by >0.5 AU) in 94% of 128 trials, peak expiratory EMG in 66%, and tonic EMG in 64%. Figure 1 shows the response to a single-step change in NUAP of 0 to −10 cmH₂O in one dog.

Compared with control values, peak inspiratory palatinus EMG activity increased significantly with all levels of NUAP (P < 0.05; Fig. 2A). Peak expiratory activity also increased significantly with all levels of NUAP less than −5 cmH₂O (P < 0.05; Fig. 2B), whereas tonic activity increased with all levels of NUAP less than −10 cmH₂O (P < 0.05; Fig. 2C). Beyond −10 cmH₂O, larger decreases in NUAP (up to −40 cmH₂O) did not result in any significant (P > 0.05) further augmentation of palatinus EMG activity (Fig. 2).

Tensor veli palatini. Data for tensor MTA EMG activity were obtained in 10 dogs. During control conditions, respiratory-related phasic inspiratory tensor EMG activity was detected in six dogs, phasic expiratory activity was found in six dogs, and low-level tonic activity was present in six dogs. Application of laryngeal NUAP increased peak inspiratory tensor MTA EMG in 74% of 132 trials, peak expiratory EMG in 30%, and tonic EMG in 32%.

Compared with control values, peak inspiratory (but not peak expiratory or tonic) tensor veli palatini EMG activity increased significantly with all levels of NUAP less than −25 cmH₂O (P < 0.05; Fig. 3). Beyond −25 cmH₂O, larger decreases in NUAP (up to −40 cmH₂O) did not result in any significant (P > 0.05) further augmentation of tensor EMG activity (Fig. 3).

Levator veli palatini. Data for levator MTA EMG activity were obtained from nine dogs. During control conditions, respiratory-related phasic inspiratory tensor EMG activity was detected in six dogs, phasic expiratory activity was found in six dogs, and low-level tonic activity was present in six dogs. Application of laryngeal NUAP increased peak inspiratory tensor MTA EMG in 74% of 132 trials, peak expiratory EMG in 30%, and tonic EMG in 32%.

Compared with control values, peak inspiratory (but not peak expiratory or tonic) tensor veli palatini EMG activity increased significantly with all levels of NUAP less than −5 cmH₂O (P < 0.05; Fig. 3). Beyond −5 cmH₂O, larger decreases in NUAP (up to −40 cmH₂O) did not result in any significant (P > 0.05) further augmentation of tensor EMG activity (Fig. 3).

Levator veli palatini. Data for levator MTA EMG activity were obtained from nine dogs. During control conditions, respiratory-related phasic inspiratory levator EMG activity was detected in four dogs and phasic expiratory activity in five animals. No tonic activity was present in any dog. Application of laryngeal NUAP resulted in variable EMG responses. Peak inspiratory levator EMG increased in only 43% of 118 trials. Peak expiratory and tonic activity increased in only 9 and 10% of trials, respectively. Consequently, there was no
significant (P > 0.05) increase in levator veli palatini EMG activity with any level of NUAP applied at the larynx (Fig. 4).

Nasal NUAP Application: SLNin Intact

Data for MTA EMG activity during NUAP applied to the nose (and not transmitted to the larynx) were obtained in 9 dogs for palatinus and in 10 dogs for both levator and tensor. In all three muscles, peak inspiratory, peak expiratory, and tonic EMG activity failed to increase with the application of nasal NUAP in the vast majority (>75%) of the 100–111 trials performed. There was no significant change in EMG activity with any individual level of nasal NUAP for any muscle (P > 0.05; Figs. 1–4).

Topographical Distribution of NUAP Responses

Palatinus. When NUAP challenges (n = 189) were applied at different topographical sites within the upper airway, there was no significant change in palatinus EMG activity within both the rostral (P > 0.5) and epiglottic zones (P > 0.1). Within the subglottic zone, peak inspiratory palatinus EMG increased significantly with NUAP (P = 0.05; Fig. 5, A and B). Peak expiratory and tonic activity also showed increases with NUAP; however, these changes achieved only borderline significance (P = 0.06 and P = 0.07, respectively; Fig. 5B). When the change in EMG activity with NUAP was compared between zones, the changes in peak inspiratory and peak expiratory EMG activity in peak inspiratory, peak expiratory, and tonic EMG activity failed to increase with the application of laryngeal NUAP in the majority (>83%) of the 55 trials performed. There was no significant change in EMG activity with any individual level of laryngeal NUAP for any muscle (P > 0.05; Figs. 1–4).

Laryngeal NUAP Application: SLNin Cut

After section of the SLNin, data was obtained in six dogs for each soft palate muscle. In all three muscles, peak inspiratory, peak expiratory, and tonic EMG activity failed to increase with the application of laryngeal NUAP in the majority (>83%) of the 55 trials performed. There was no significant change in EMG activity with any individual level of laryngeal NUAP for any muscle (P > 0.05; Figs. 1–4).

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the subglottic zone were significantly greater than those in the rostral zone (P < 0.05). However, changes in the epiglottic zone were not significantly different from those in the other two zones (P > 0.05).

Tensor veli palatini. When NUAP challenges (n = 197) were applied at different topographical sites within the upper airway, changes in peak inspiratory, peak expiratory, and tonic EMG activity of the tensor veli palatini muscle were not significant within the rostral (P > 0.4) and epiglottic (P > 0.1) zones. Within the subglottic zone, peak expiratory and tonic EMG activity also did not change (P > 0.1). However, there was a significant increase in peak inspiratory activity (P = 0.04; Fig. 6, A and B). Although the changes in EMG activity with NUAP tended to be greater in the epiglottic and subglottic zones (Fig. 6B), a significant difference (P > 0.1) between the zones was not detected.

Levator veli palatini. When NUAP challenges (n = 183) were applied at different topographical sites within the upper airway, there was no significant change in levator veli palatini EMG activity within any zone (P > 0.08; Fig. 7, A and B). However, when the change in EMG activity was compared between zones, the change in peak inspiratory EMG activity (but not peak expiratory or tonic activity) was significantly greater in the subglottic zone than in the other two zones (P < 0.05, Fig. 7).

**DISCUSSION**

The principal findings in this study are that in anesthetized, supine, tracheostomized, mouth-closed dogs 1) tensor veli palatini and palatinus muscle EMG activity is augmented by negative pressure in the hypopharynx, larynx, and cranial trachea, 2) levator veli palatini muscle EMG activity is weakly and inconsistently recruited by laryngeal NUAP, 3) NUAP applied exclusively to the nasal passages or nasopharynx does not recruit soft palate muscle EMG activity, 4) recruitment of soft palate muscle activity with NUAP is mediated via the SLNin, and 5) the reflex activation of soft palate muscles with NUAP is maximal with a NUAP level of −10 cmH₂O. In addition, it was observed that in most dogs the upper airway remained closed during the application of NUAP despite maximal...
NUAP-mediated reflex recruitment of soft palate muscle activity.

Application of NUAP to the laryngeal end of the upper airway (and not transmitted to the nose) consistently resulted in the recruitment of palatinus and tensor veli palatini muscle EMG activity (see Figs. 1–3). This augmentation of EMG activity occurred on the first breath after the change in NUAP (see Fig. 1). Incomplete adaptation was observed with maintenance of the stimulus being associated with a fall in response compared with the first breath after application of NUAP (see Fig. 1). This phenomenon has been reported previously for other upper airway muscles (13) and also in terms of the discharge of receptors (9).

The responses of the palatinus and tensor veli palatini muscles to laryngeal NUAP in the present study are in general agreement with our previous findings in dogs in which only a single level of NUAP was used (22) and with those of Wheatley et al. (25) in humans. However, the lack of consistent recruitment of the levator veli palatini by laryngeal NUAP was surprising in view of previous results demonstrating responses of the levator to NUAP in humans (19) and animals (22). The reason for this discrepancy is not entirely clear. However, even in our previous study, some individual animals did not show a levator response to NUAP (22).

In the present study, levator responses to NUAP were small and variable. Because general anesthesia is known to depress the recruitment of upper airway muscles (7), the effect of anesthesia on levator activity is a concern; however, α-chloralose produces light, stable anesthesia, and all soft palate muscles within each animal were studied concurrently. Consequently, for the depressant effects of anesthesia to have been responsible for the lack of recruitment of levator, this would have had to have been a selective effect for this muscle compared with the other soft palate muscles. Thus the levator veli palatini in the dog appears to be only inconsistently and weakly recruited in response to NUAP applied at the larynx. This may also be the case in humans because levator veli palatini EMG activity in humans has been shown to have high inter- and intrasubject variability both in terms of its activity during quiet breathing and in its response to chemostimulation (12).

The complete loss of the response to laryngeal NUAP after bilateral section of the SLN in is consistent with results reported for the response of other upper airway muscles to laryngeal NUAP (13, 15, 23) and establishes that the SLN is also the afferent pathway for soft palate muscle recruitment.

When the NUAP was applied exclusively to the nasal passages (and not transmitted to the larynx), there was no augmentation of soft palate muscle EMG activity. This finding suggests that local negative pressure reflex control of soft palate muscle activity does not involve receptors located in the nose. This conclusion for the soft palate muscles is in contrast to results reported for the genioglossus in rabbits, where nasal NUAP (not transmitted to the larynx) has been shown to recruit genioglossus EMG activity (15). In dogs, the application of NUAP to the nasal cavity has been shown to augment alae nasi and posterior cricoarytenoid muscle activity in some studies (23, 26), whereas other studies have attributed the response to NUAP almost entirely to laryngeal afferents (11, 16). Furthermore, Kaminuma and co-workers (11) reached the conclusion that nasal pressure receptors have only minimal impact on the reflex recruitment of posterior cricoarytenoid muscle activity in dogs. The present study now extends this conclusion to the canine soft palate muscles.

Some previous studies have suggested that the oropharynx is also not an important site for NUAP-related reflex recruitment of genioglossus muscle activity (2). Moreover, sectioning of the glossopharyngeal nerves in cats increases rather than decreases the augmentation of hypoglossal nerve activity with NUAP (8). Results from the present study suggest that the pharynx is also not important in the NUAP-mediated reflex recruitment of the soft palate muscles.

In the present study, laryngeal afferent activity appeared to be the only pathway for augmentation of soft palate muscle activity by NUAP. We base this conclusion on the following results: 1) the response was not elicited by nasal NUAP, 2) the response disappeared after sectioning of the SLN, and 3) the response was confined to the epiglottic/laryngeal region of the upper airway in the group 2 dogs. However, evidence obtained in laryngectomized humans, in whom afferent impulses from the larynx have been ablated, suggests that genioglossus muscle EMG activity is still augmented by NUAP (10), an effect attributed to receptors located in the oropharyngeal and/or nasopharyngeal mucosa. This evidence suggests that, at least in humans, pharyngeal receptors do exist and may play an enhanced role in the absence of laryngeal receptor input. However, in the intact upper airway it seems that it is the laryngeal receptors which play the dominant role.

The stimulus response characteristics for soft palate muscle activation by graded NUAP have not been previously studied over the range of NUAP employed in the present study. The finding that there was no further augmentation of soft palate muscle EMG activity by NUAP beyond −10 cmH₂O is similar to previously reported data for other upper airway muscles (4, 17, 23). The plateau of reflex recruitment might be interpreted as representing maximal muscle EMG activation. However, we have previously observed spontaneous levels of soft palate muscle EMG activity during upper airway breathing in anesthetized dogs (22) that were two to four times higher than those measured at an equivalent level of NUAP (−11.3 ± 1.8 cmH₂O) to that associated with the maximum EMG recruitment in the present study. Plateauing of upper airway muscle responses to NUAP may then suggest saturation of receptor activation, with muscle EMG recruitment via this reflex pathway limited to submaximal levels. Indeed, discharge frequencies of receptors generating changes in afferent activity in the SLN in response to NUAP have been shown to reach plateau levels in the −7- to −28-cmH₂O range in cats (9).
In conclusion, we have demonstrated that, in supine, anesthetized dogs, reflex recruitment of soft palate muscle activity by NUAP is predominantly modulated by afferent activity originating from the laryngeal and/or hypopharyngeal airway and transmitted via the SLN. The soft palate muscles may exhibit selective reflex recruitment in response to NUAP in that the levator frequently failed to respond to NUAP, whereas the palatopharyngeus and, to a lesser extent, tensor both consistently increased their activity. The response of the soft palate muscle activity in humans. J. Appl. Physiol. 70: 2574–2581, 1991.


