Superior laryngeal nerve section alters responses to upper airway distortion in sleeping dogs

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Curran, Aidan K., Peter R. Eastwood, Craig A. Harms, Curtis A. Smith, and Jerome A. Dempsey. Superior laryngeal nerve section alters responses to upper airway distortion in sleeping dogs. J. Appl. Physiol. 83(3): 768–775, 1997.—We investigated the effect of superior laryngeal nerve (SLN) section on expiratory time (TE) and genioglossus electromyogram (EMGgg) responses to upper airway (UA) negative pressure (UANP) in sleeping dogs. The same dogs used in a similar intact study (C. A. Harms, C. A., Y.-J. Zeng, C. A. Smith, E. H. Vidruk, and J. A. Dempsey. J. Appl. Physiol. 80: 1528–1539, 1996) were bilaterally SLN sectioned. After recovery, the UA was isolated while the animal breathed through a tracheostomy. Square waves of negative pressure were applied to the UA from below the larynx or from the mask (nares) at end expiration and held until the next inspiratory effort. Section of the SLN increased eupneic respiratory frequency and minute ventilation. Relative to the same dogs before SLN section, sublaryngeal UANP caused less TE prolongation while activation of the genioglossus required less negative pressures. Mask UANP had no effect on TE or EMGgg activity. We conclude that the SLN 1) is not obligatory for the reflex prolongation of TE and activation of EMGgg activity produced by UANP and 2) plays an important role in the maintenance of UA stability and the pattern of breathing in sleeping dogs.

METHODS
Animal Instrumentation

The three adult female mixed-breed dogs used in this study were the same dogs used previously by Harms et al., and thus the surgical implantation of chronic instrumentation has already been described. Briefly, under general anesthesia (premedication with 0.5 mg/kg acepromazine, induction with 20 mg/kg pentobarbital sodium iv, maintained with 1% halothane in O2), the animals had surgically implanted permanent tracheostomies, electromyogram (EMG) electrodes in the crural diaphragm and GG muscles, as well as a five-lead electroencephalogram (EEG)-electrooculogram montage for the purpose of sleep staging during experiments. An analgesic (0.3 mg/kg butorphanol) and an antibiotic (15 mg/kg trimethaprim-sulfa) were administered postoperatively as required.

For the purpose of this study, similar techniques were used to perform a second sterile surgery to denervate the SLNs. A ventral midline incision was made in the neck, and the SLNs were exposed bilaterally and cut close to their insertion on the vagus nerves. At least 2 wk were allowed for recovery before experiments. SLN section was confirmed before commencement of experiments by the absence of a gag reflex due to mechanical probing of the larynx and the instillation of saline or distilled water into the larynx via the sublaryngeal catheter (Fig. 1). The surgical and experimental protocols of this activity in response to negative pressure applied to the isolated UA at end expiration in awake and sleeping dogs. The prolongation of TE became apparent only after the airway collapsed and increased as more negative pressure was applied to the collapsed airway. This effect was independent of whether the pressure was applied from below the larynx or from the face mask. This indicates that the SLN may not be the primary afferent arm of this reflex because the pressure applied to the UA via the mask would not be transmitted to the larynx after UA collapse, yet the prolongation of TE was still correlated with the applied negative pressure. Also, because topical anesthesia of the UA, which abolished all responses to water and ammonia, failed to abolish the response, Harms et al. reasoned that, because surface-receptor output had been abolished, it was the distortion of deeper structures in the UA after collapse had occurred that produced the responses seen. There is evidence that the SLN contains fibers that innervate deeper structures in the vicinity of the epiglottis (26). Such receptors provide a potential pathway for the response to UANP and would likely be unaffected by topical airway anesthesia. The purpose of the present study was to examine what role, if any, the SLN plays in the reflex responses to UANP as reported by Harms et al.

Upper Airway (UA) Negative Pressure (UANP) has been shown to alter the pattern of breathing in anesthetized dogs (4, 29) and rabbits (14, 15) and in awake and sleeping dogs (5, 8, 16). In general, these effects were a prolongation of inspiratory duration (TI), although a substantial number of receptors sensitive to airway pressure (2, 24). Section of the SLN has been shown to reduce or abolish the inhibition of breathing due to UANP applied to the larynx in anesthetized dogs (29) and rabbits (15) and to reduce or abolish the EMGgg activation secondary to UANP in anesthetized dogs (29) and rabbits (11, 12, 15).

Recently, Harms et al. (5) demonstrated a substantial prolongation of TE and an increase in tonic EMGgg activity in response to negative pressure applied to the isolated UA at end expiration in awake and sleeping dogs. The prolongation of TE became apparent only after the airway collapsed and increased as more negative pressure was applied to the collapsed airway. This effect was independent of whether the pressure was applied from below the larynx or from the face mask. This indicates that the SLN may not be the primary afferent arm of this reflex because the pressure applied to the UA via the mask would not be transmitted to the larynx after UA collapse, yet the prolongation of TE was still correlated with the applied negative pressure. Also, because topical anesthesia of the UA, which abolished all responses to water and ammonia, failed to abolish the response, Harms et al. reasoned that, because surface-receptor output had been abolished, it was the distortion of deeper structures in the UA after collapse had occurred that produced the responses seen. There is evidence that the SLN contains fibers that innervate deeper structures in the vicinity of the epiglottis (26). Such receptors provide a potential pathway for the response to UANP and would likely be unaffected by topical airway anesthesia. The purpose of the present study was to examine what role, if any, the SLN plays in the reflex responses to UANP as reported by Harms et al.
Behavior was monitored by using a closed-circuit television camera.

Experimental Protocol

Isolation of the UA

The dogs were intubated with a cuffed tracheostomy tube (12.0 mm OD) via the tracheostomy (Fig. 1). The tracheostomy tube was connected to a heated pneumotachograph and pressure transducer system (Hans Rudolph 3700, Kansas City, MO; Validyne MP-45-14-871, Northridge, CA). Calibration was performed before each experiment by using five known flow rates. EMGgg and costal diaphragm EMG activation was performed before each experiment by using five known flow rates. EMGgg and costal diaphragm EMG activities were amplified, rectified, and moving time averaged with a time constant of 100 ms (CWE, Ardmore, PA).

Isolation of the UA

The UA was isolated by sealing both around the mask and below the larynx (Fig. 1). A tight-fitting lightweight plastic face mask was fitted to the dogs face and lined with a gel-polymer sheath that provided an airtight seal around the dog's snout. This mask ensured that the dogs were unable to open their mouths, and thus UANP applied at the mask was transmitted to the UA only via the nose. A balloon-tipped catheter was then inserted rostrally into the tracheostomy tube to lie just caudal to the cricoid cartilage. Inflation of the balloon produced an isolated UA (Fig. 1). This catheter as well as a second catheter inserted through the face mask to lie near the nares were connected to pressure transducers (Validyne) and used to simultaneously measure mask and sublaryngeal pressures. These transducers were calibrated before each experiment by application of eight known pressures. Application of negative pressure to the isolated UA was achieved by using a syringe connected to either the sublaryngeal or the mask catheter. The dogs’ behavior was monitored by using a closed-circuit television camera.

Once a stable breathing pattern was observed during quiet wakefulness or non-rapid-eye-movement (NREM) sleep, UANP trials were performed with at least a 2-min interval between each trial. Trials consisted of the application of a square wave of negative pressure to the isolated UA via either the sublaryngeal catheter or the mask catheter late in expiration and the maintenance of the pressure until the beginning of the next inspiratory effort (Fig. 2). Multiple trials were performed with pressures ranging from −1 to −32 cmH2O.

Sleep Staging

Standard canine sleep staging criteria were used to identify sleep stage (22). NREM sleep was defined as a slow-wave EEG without associated rapid eye movements. An EEG arousal was defined as desynchronous EEG activity for >3 s. Any trials where there was evidence of arousal were excluded from further analysis.

UA Closure

During trials in sleep, the pressure at which the mask and sublaryngeal pressures diverged during the application of UANP was defined as the closing pressure (Fig. 2). To estimate the site of UA closure, a terminal study was performed in two of the dogs. After anesthesia with pentobarbital sodium, the dogs were instrumented for application of UANP as described in Ventilation and EMGs. In addition, a third catheter was inserted through the nose and passed down to the level of the larynx. This catheter was also connected to a pressure transducer (Validyne). Application of negative pressure from either the mask or sublaryngeal catheter could be measured in the third catheter. This catheter was withdrawn 1 cm at a time, and collapsing pressures were applied from the mask and sublaryngeal catheters. By measuring the resultant pressure deflection in the third catheter and noting any deviation from the pressure applied, we were able to determine whether this catheter was in communication with the area above or below the site of collapse. By this method we were able to estimate the site within the UA at which collapse occurred in each case.

Data Collection and Analysis

All signals were recorded on a 12-channel polygraph (Gould ES 2000, Rolling Meadows, IL) and were passed through an analog-to-digital converter and stored on the hard disk of a microcomputer for later analysis using a software package developed in house. All signals were sampled at 64 Hz. The software calculated ventilatory variables breath by breath from the flow signal. Each dog acted as its own control. For the purpose of statistical comparison, the data presented here for the SLN-intact dogs are redrawn from the data of Harms et al. (5). Statistical comparisons of the eupneic values for ventilatory variables for and the closing pressures between SLN-intact and SLN-sectioned dogs were performed by using unpaired Student's t-tests. Analysis of covariance was used to examine the effect of UANP on Tc between SLN-intact and SLN-sectioned dogs as well as between UANP applied at the mask and from below the larynx. Differences between groups were tested by using both linear and quadratic terms. In all cases, P < 0.05 was considered significant.

RESULTS

NREM

Effect of SLN section on eupneic ventilation. Section of the SLN produced a marked alteration of the pattern

Fig. 1. Schematic representation of isolated upper airway (UA) preparation. Psub-laryngeal, Psub-laryngeal, and Psub-laryngeal, and mask pressures, respectively; EOG, electrooculogram; EEG, electroencephalogram; Pneumotach, pneumotachograph.

Ventilation and EMGs

The dogs were trained to sleep on a bed in an air-conditioned, sound-attenuated chamber while wearing the face mask and tracheostomy apparatus. Dogs were unrestrained and allowed to choose their own posture. The dogs' behavior was monitored by using a closed-circuit television camera.
of eupneic ventilation in all three dogs during NREM sleep (Table 1). After section, control values for respiratory frequency and minute ventilation increased significantly in all three dogs (27 and 31\% for respiratory frequency and minute ventilation, respectively, for the 3 dogs combined). The effect on respiratory frequency was due to reductions in both inspiratory and expiratory durations (−30 and −12\% respectively). Tidal volume was generally unaffected, with only dog 2 showing a significant increase.

Effect of SLN section on the Te response to UANP. UANP APPLIED FROM BELOW THE LARYNX. An example of the response to UANP applied from below the larynx after SLN section is shown in Fig. 2A. Notice the fall in both mask and sublaryngeal pressures during the initial phase of the pressure application and the plateau in the mask pressure at approximately −4 cmH₂O. This plateau signifies that the pressure being applied from below the larynx is no longer being transmitted to the mask, indicating that the UA has collapsed. Notice also after the collapse the prolongation of TE and the increase in the level of tonic EMGgg activity.

Application of negative pressure to the isolated UA from below the larynx during NREM sleep in SLN-intact dogs at end expiration produced a marked prolongation of TE. This increase in TE was dose dependent with a threshold for prolongation at approximately −8 cmH₂O and a maximal effect at −30 cmH₂O (Fig. 3). The term threshold refers to the fact that application of pressures above −8 cmH₂O in the SLN-intact dogs failed to produce a response, whereas more negative pressures caused TE prolongation. After SLN section, application of negative pressure to the UA from below the larynx still caused a significant increase in TE in all

Table 1. Raw values for ventilatory variables for each dog during NREM sleep before and after section of SLN

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>SLN</th>
<th>n</th>
<th>f, breaths/min</th>
<th>Tᵢ, s</th>
<th>Tₑ, s</th>
<th>Vᵣ, liters</th>
<th>Vᵢ, l/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact</td>
<td>75</td>
<td>9.8 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>4.5 ± 0.1</td>
<td>0.35 ± 0.04</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sectioned</td>
<td>32</td>
<td>11.9 ± 0.3*</td>
<td>1.6 ± 0.02</td>
<td>3.8 ± 0.1*</td>
<td>0.35 ± 0.01</td>
<td>4.1 ± 0.1*</td>
</tr>
<tr>
<td>2</td>
<td>Intact</td>
<td>84</td>
<td>9.0 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>0.26 ± 0.01</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sectioned</td>
<td>64</td>
<td>9.7 ± 0.1*</td>
<td>1.8 ± 0.02*</td>
<td>4.5 ± 0.1*</td>
<td>0.30 ± 0.01*</td>
<td>2.9 ± 0.1*</td>
</tr>
<tr>
<td>3</td>
<td>Intact</td>
<td>85</td>
<td>9.8 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>0.32 ± 0.01</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Sectioned</td>
<td>45</td>
<td>14.8 ± 0.4*</td>
<td>1.3 ± 0.03*</td>
<td>3.0 ± 0.1*</td>
<td>0.31 ± 0.01</td>
<td>4.7 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of trials. SLN, superior laryngeal nerve; f, respiratory frequency; Tᵢ and Tₑ, inspiratory and expiratory duration, respectively; Vᵣ, tidal volume; Vᵢ, minute ventilation. *Significantly different from SLN intact, P < 0.05 (Student’s t-test).
three dogs during NREM sleep. After section of the SLN, a threshold for \( T_E \) prolongation was not as evident as in the intact animals because of the greater scatter in the data. In each dog, however, the response to UANP applied from below the larynx was slightly but significantly less than that seen in the same animal before section of the SLN (Fig. 3).

**UANP APPLIED FROM THE MASK.** An example of the response to UANP applied at the mask after SLN section is shown in Fig. 2B. Notice that, in this case, UA collapse resulted in a plateau in the sublaryngeal pressure trace at approximately \(-4 \, \text{cmH}_2\text{O}\) and that there was no effect on Te or EMGgg activity.

The effect of UANP applied at the mask is shown in Fig. 3. When UANP was applied from the mask during NREM sleep in the SLN-intact dogs, Te was increased to the same extent as that seen with UANP applied from below the larynx. However, after SLN section, there was no effect of UANP applied at the mask on Te in any of the three dogs.

**Effect of SLN section on the GG response to UANP.** An example of the activation of the GG by UANP is shown in Fig. 2A. The response of the GG to UANP is shown for all dogs in Table 2. In the SLN-intact animals the incidence of GG activation by UANP was greater as the negative pressure applied became more negative. This effect was independent of the route of application of the negative pressure. After SLN section, EMGgg activity was still increased by UANP applied from below the larynx during NREM sleep. The pattern of activation was similar to that reported for the SLN-intact dogs, namely the greater the negative pressure applied the greater the frequency of GG activation. Notice, however, that after SLN section there was a greater incidence of GG activation in the pressure range between \(-4 \) and \(-15 \, \text{cmH}_2\text{O}\) (14, 25, and 0% of trial for each dog with intact SLN vs. 33, 75, and 100% after section, respectively).

After SLN section, when UANP was applied from the mask during NREM sleep, there was no GG activation in any of the three animals. This is in sharp contrast to the effect of SLN section on the response to UANP applied from below the larynx.

**Effect of SLN section on airway closing pressure and site of closure.** The effect of SLN section on the pressure at which UA collapse occurred is shown in Fig. 4. In SLN-intact dogs, application of UANP from below the larynx produced UA collapse at a negative pressure of approximately \(-8 \, \text{cmH}_2\text{O}\) during NREM sleep. This was not significantly different from the collapsing pressure when the UANP was applied from the mask (Fig. 4). After section of the SLN, the collapsing pressure was approximately \(-4 \, \text{cmH}_2\text{O}\) (Figs. 2, A and B, and 4), with no significant difference between the

### Table 2. Effect of upper airway negative pressure on genioglossus muscle activation

<table>
<thead>
<tr>
<th>Upper Airway Negative Pressure, cmH(_2)O</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SLN intact</td>
<td>SLN sectioned</td>
<td>SLN intact</td>
</tr>
<tr>
<td></td>
<td>(P_u)</td>
<td>(P_m)</td>
<td>(P_u)</td>
</tr>
<tr>
<td>0 to (-4)</td>
<td>0/12 0/2</td>
<td>0/1 0/4</td>
<td>0/15 1/11</td>
</tr>
<tr>
<td>(-5) to (-14)</td>
<td>2/14 3/7</td>
<td>1/3 0/6</td>
<td>3/12 4/8</td>
</tr>
<tr>
<td>(-15) to (-24)</td>
<td>16/17 6/6</td>
<td>1/3 0/5</td>
<td>19/19 4/4</td>
</tr>
<tr>
<td>(-24) to (-32)</td>
<td>14/14 3/3</td>
<td>5/8 0/2</td>
<td>17/17 8/8</td>
</tr>
</tbody>
</table>

Values are number of trials in which genioglossus muscle activated because of application of upper airway negative pressure applied from below larynx [sublaryngeal pressure \(P_u\)] with SLN intact and SLN sectioned or from the mask [mask pressure \(P_m\)] with SLN intact or sectioned as a fraction of total number of trials in each group of pressure trials for each dog. Prevalence of activation because of upper airway negative pressure from below the larynx after SLN section is similar to that reported for SLN-intact dogs. Note, however, that when pressure is applied from mask after SLN section, there is no activation of genioglossus.
In two of the dogs, the area in the UA where closure occurred due to UANP was measured under anesthesia by using a third, moveable airway catheter (see Methods). In these trials a negative pressure of approximately −20 cmH₂O was applied to the UA to ensure collapse. Collapse occurred at approximately −4 cmH₂O (or 20% of the pressure applied). Pressures were applied from below the larynx and from the mask. The results are shown schematically in Fig. 5, A and B. These results indicate that the nasopharynx collapsed over a region measuring 5–7 cm stretching from the base of the soft palate rostrally toward the base of the hard palate (Fig. 5A). This is a substantially larger area than that reported for one of the dogs before section of the SLN (5), where the site of collapse collapsing pressure between UANP applied at the mask or from below the larynx.

In two of the dogs, the area in the UA where closure occurred due to UANP was measured under anesthesia by using a third, moveable airway catheter (see Methods). In these trials a negative pressure of approximately −20 cmH₂O was applied to the UA to ensure collapse. Collapse occurred at approximately −4 cmH₂O (or 20% of the pressure applied). Pressures were applied from below the larynx and from the mask. The results are shown schematically in Fig. 5, A and B. These results indicate that the nasopharynx collapsed over a region measuring 5–7 cm stretching from the base of the soft palate rostrally toward the base of the hard palate (Fig. 5A). This is a substantially larger area than that reported for one of the dogs before section of the SLN (5), where the site of collapse
appeared to be over a region not larger than 2 cm, stretching from the base of the soft palate upward (see Fig. 5A).

Wakefulness

Application of UANP during wakefulness was only possible in two of the dogs after SLN section because of the overt behavioral responses of dog 1 to application of any pressure more negative than −10 cmH2O. During wakefulness in the other two dogs, results were very variable. Both dogs showed TE prolongation and EMGg activity in response to UANP applied from below the larynx of similar magnitude to that reported during sleep. Application of UANP from the mask during wakefulness, however, produced a TE prolongation similar to that seen with UANP applied from below the larynx, while only one of the dogs showed an increase in EMGg activity with UANP applied at the mask.

Because of these variations and the overt behavioral effects, we do not believe that the use of the awake state permitted us to properly evaluate the effect of SLN section per se on the effect of UANP on breathing and EMGg activity.

It may be the case that SLN section produced a more prevalent behavioral response during wakefulness because of some conscious sensation associated with collapse of the airway over a larger area.

DISCUSSION

The main findings from this study are as follows. 1) Section of the SLN alters the pattern of breathing in tracheostomized, sleeping dogs by means of an increase in respiratory frequency and minute ventilation. 2) The SLN is not required for either the TE prolongation or the increase in EMGg activity produced by UANP applied from below the larynx in sleeping dogs, because the response is still present after section. 3) Section of the SLN, however, does lead to the abolition of both the TE prolongation and the EMGg activation due to UANP applied at the mask during NREM sleep. This is likely because section of the SLN produced a more widespread UA collapse such that UANP applied at the mask failed to stimulate the presumably more caudal receptive field responsible for both the TE prolongation and the increase in EMGg activity.

Effect of SLN Section on Eupneic Ventilation

The effect of SLN section on eupneic ventilation has been studied in conscious adult rats (19), neonatal kittens (20), and neonatal guinea pigs (3). In the conscious rats and kittens, the authors reported an increase in respiratory frequency and minute ventilation with no effect on tidal volume (3). This difference may reflect a different role of the SLN in the neonatal vs. adult animals.

It is important to note that the dogs in this study were breathing via a tracheostomy and thus were without most of the normal feedback from UA afferents in the SLN even during the SLN-intact experiments. Accordingly, the feedback from temperature receptors and other receptors active during eupneic airflow were absent, and the only afferent activity in the SLN would be from “driving receptors” (24), which are responsive to mechanical stimuli such as tracheal tug (27) and which would still provide some feedback in the absence of airflow. Such receptors have been described as naked nerve endings in the airway muscle; they act like spindles (10) and have activity that can persist even after topical airway anesthesia (9). The absence of this feedback after SLN section would appear to be the source of the alteration in the pattern of breathing after SLN section. Indeed, this is consistent with the lack of effect of topical airway anesthesia on the pattern of breathing in the intact dogs (5) which would only affect the surface receptors. The increase in respiratory frequency after SLN section may reflect an inhibitory effect of these deeper mechanoreceptors on eupneic ventilation. Increasing the activity of these receptors by negative pressure application may then be expected to inhibit further, leading to an apnea. The reduction in the TE prolongation in response to airflow distortion after SLN section is likely to be due to loss of the afferent innervation of these deeper receptors. The remaining TE prolongation and EMGg activation after SLN section is likely to be due to similar receptors elsewhere in the airway that are carried in other nerves such as the glossopharyngeal or trigeminal nerves, the relative importance of which remains to be determined.

One might expect that SLN section in animals breathing through the UA would have a much larger effect on the pattern of eupneic breathing because there would be even greater SLN activity associated with eupneic airflow. Similarly, the SLN, although not obligatory for the TE prolongation and EMGg activation associated with UANP, plays an important role in the regulation of the pattern of eupneic ventilation through the presence of a respiratory-related feedback.

Effect of SLN section on EMGg Activity and UA Closure

SLN section has been shown to abolish the effect of UANP applied from below the larynx on EMGg activity in the anesthetized rabbit (11). After SLN section, EMGg activation due to UANP applied from below the larynx was still present but was absent when UANP was applied at the mask. The difference in the results between this study and the rabbit study may be due to the presence of anesthesia in the rabbit model, because anesthesia has been shown to reduce or abolish UA reflexes (6).

UANP has also been shown to increase hypoglossal nerve activity in decerebrate, paralyzed, ventilated
The presence of the TE prolongation and EMG activation reflexes during the expiratory phase may be of interest considering data in sleeping humans that demonstrate that the airway can close even during a central apnea (1). The airway distortion associated with this collapse may act to prolong the apnea. Indeed, recent evidence has shown that the airway narrows progressively during the expiratory phase in the breaths leading to a central apnea (17, 18). This progressive inspiratory narrowing in the absence of UANP is associated with increasing expiratory durations (23) and may be part of a cycle of airway wall stretch due to passive airway narrowing leading to longer expiratory durations, which allow further narrowing ultimately leading into an apnea.

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