Superior laryngeal and hypoglossal afferents tonically influence upper airway motor excitability in anesthetized rats

Stephen Ryan and Philip Nolan

Departments of Human Anatomy and Physiology, Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

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Ryan, Stephen, and Philip Nolan. Superior laryngeal and hypoglossal afferents tonically influence upper airway motor excitability in anesthetized rats. J Appl Physiol 99: 1019–1028, 2005; doi:10.1152/japplphysiol.00776.2004.—Upper airway (UA) muscle activity is stimulated by changes in UA transmural pressure and by asphyxia. These responses are reduced by muscle relaxation. We hypothesized that this is due to a change in afferent feedback in the ansa hypoglossi and/or superior laryngeal nerve (SLN). We examined 1) the glossopharyngeal motor responses to UA transmural pressure and asphyxia and 2) how these responses were changed by muscle relaxation in animals where one or both of these afferent pathways had been sectioned bilaterally. Experiments were performed in 24 anesthetized, thoracotomized, artificially ventilated rats. Baseline glossopharyngeal activity and its response to UA transmural pressure and asphyxia were moderately reduced after bilateral section of the ansa hypoglossi (P < 0.05). Conversely, bilateral SLN section increased baseline glossopharyngeal activity, augmented the response to asphyxia, and abolished the response to UA transmural pressure. Muscle relaxation reduced resting glossopharyngeal activity and the response to asphyxia (P < 0.001). This occurred whether or not the ansa hypoglossi, the SLN, or both afferent pathways had been interrupted. We conclude that ansa hypoglossi afferents tonically excite and SLN afferents tonically inhibit UA motor activity. Muscle relaxation depressed UA motor activity after section of the ansa hypoglossi and SLN. This suggests that some or all of the response to muscle relaxation is mediated by alterations in the activity of afferent fibers other than those in the ansa hypoglossi or SLN.

Address for reprint requests and other correspondence: P. Nolan, Michael Tierney Bldg., Univ. College Dublin, Earlsfort Terrace, Dublin 2, Ireland (E-mail: philip.nolan@ucd.ie).

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Surgical anesthesia was induced with pentobarbitone sodium (60 mg/kg ip; Sagatal, Rhone Merieux) and maintained with intravenous infusion of alphaxalone-alphadalone acetate (4–8 mg·h⁻¹·ml⁻¹ iv; Saffan) to maintain a stable systemic arterial pressure and respiratory rate as well as to suppress reflex withdrawal, arterial pressure, and respiratory responses to paw pinch. The rats were placed in the supine position on a thermostatically controlled heating blanket (homeothermic blanket system, Harvard Instruments, Kent, UK) to maintain rectal temperature close to 37°C. The right femoral artery and vein were cannulated for recording of systemic arterial pressure (Statham P23Dd, Hato Rey, PR) and for injection of drugs, respectively.

Animals were tracheostomized, preserving the recurrent laryngeal nerves, thoracotomized, and artificially ventilated (model CWE SAR-830/P, Linton Instrumentation, Norfolk, UK; fraction of inspired O₂ = 0.33, respiratory rate = 90 breaths/min, inspiratory time = 300 ms, tidal volume = 1–2 ml, positive end-expiratory pressure = 1–2 cmH₂O). The ventilator was modified so that lung inflation occurred in response to phrenic nerve activity. A rectified, moving-time-averaged phrenic electroneurogram signal was processed by a custom-designed interface that detected the onset of phrenic nerve activity and also its sharp decline at the beginning of the postinspiratory period. The interface generated electronic pulses marking these events, and these pulses were used to control the ventilator. The system was set so that when the onset of inspiratory phrenic nerve activity was detected, the ventilator began delivery of a constant inspiratory airflow (6–9 ml/s; inspired O₂ fraction = 0.33) to the lungs, which was terminated on detection of the onset of postinspiration (typical tidal volume = 2–3 ml). A differential pressure transducer within the ventilator continuously monitored tracheal pressure.

**UA preparation.** Atropine sulfate (0.1 mg/kg iv; Antigen Pharmaceuticals) was administered to reduce UA secretions. The UA was exposed to changes in transmural pressure, as we previously described (23). Briefly, the UA was isolated after a second cannula was inserted in the trachea, pointing cranially, with its tip lying ~5 mm below the vocal cords. A tight-fitting plastic mask was applied to the snout, and an airtight seal was ensured using petroleum jelly. The laryngeal cannula and mask were connected together and then to a pressure reservoir via a solenoid valve. The pressure reservoir could be evacuated or pressurized to a predetermined level in the range ~10 to ~20 cmH₂O, so that activation of the solenoid valve allowed controlled application of that pressure to the isolated UA segment. We have found that subatmospheric pressure applied to the subglottic cannula alone causes closure of the glottis or collapse of the pharynx in an unpredictable fashion. However, if suction is applied simultaneously to the nasal and laryngeal ends of the airway in a leak-free system, then laryngeal and pharyngeal closure do not occur, and a reproducible transmural pressure change occurs throughout the entire

**Table 1. Arterial blood gases before and after afferent and motor nerve section and muscle relaxation**

<table>
<thead>
<tr>
<th></th>
<th>( P_{aCO_2} ), Torr</th>
<th>( P_{aO_2} ), Torr</th>
<th>( pH )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>42.3 ± 1</td>
<td>151.9 ± 4.5</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>Pre-NMB</td>
<td>43.1 ± 1.2</td>
<td>158.7 ± 4.6</td>
<td>7.38 ± 0.015</td>
</tr>
<tr>
<td>Post-NMB (pancuronium)</td>
<td>42.9 ± 0.8</td>
<td>162.6 ± 5.1</td>
<td>7.35 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( P_{aCO_2}, P_{aO_2}, \) and \( pH \), arterial PCO₂, PO₂, and pH; NMB, neuromuscular blockade.

Fig. 1. Glossopharyngeal activity and its response to upper airway (UA) negative pressure (UANP) after section of the ansa hypoglossi and superior laryngeal nerve (SLN) in anesthetized rat. Original records show effect of ~20-cmH₂O UA transmural pressure on glossopharyngeal motor nerve activity (PH-IX) in the intact state (A), after bilateral ansa hypoglossi section (B), after subsequent bilateral SLN section (C), and after neuromuscular blockade with pancuronium (D).
length of the UA (24). Pressures were therefore applied simultaneously to the mask and the laryngeal cannula. A transducer attached to the laryngeal cannula monitored the pressure applied to the UA in all experiments (±35 cmH₂O; model DP45, Validyne, Northridge, CA).

Nerve recording. All neural recordings were obtained using glass suction electrodes filled with phosphate-buffered saline solution (0.01 M; Sigma Aldrich). Electrodes were pulled using a Narshige electrode puller (model PC-10) and broken back until the bore diameter matched that of the cut end of the nerve (40–80 μm, depending on the nerve). Activity was amplified (Neurolog NL100AK preamplifier and NL104 amplifier, Digitimer, Welwyn Garden City, UK), filtered (0.3–2 kHz; Neurolog NL125), processed by a leaky integrator (time constant = 100 ms; Neurolog NL703), and fed to an audio monitor and an oscilloscope.

Neural recordings of the right glossopharyngeal and phrenic motor nerves were recorded in all animals (n = 24). The glossopharyngeal nerve was sectioned distal to the carotid sinus branch. Neural signals, together with systemic arterial pressure, tracheal pressure, UA pressure, and UA airflow were recorded and stored on a computer using a Micro1401 interface and Spike 2 software (CED, Cambridge, UK).

Experimental protocols. Glossopharyngeal motor activity was recorded at rest (0-cmH₂O UA transmural pressure) and on the first breath after changes in UA transmural pressure (+10 and −20 cmH₂O) applied for 5 s while ventilation continued. The response to brief progressive asphyxia (ventilator stopped for 20 s with lungs collapsed against the applied positive end-expiratory pressure) was also examined.

First, a series of experiments (n = 12) were conducted to examine the effect of sectioning the ansa hypoglossi or SLN alone, followed by muscle relaxation, on glossopharyngeal motor activity and its responses to UA transmural pressure and asphyxia. Responses were recorded first with all afferent pathways intact and then after section of the ansa hypoglossi (6 experiments) or the SLN alone (6 experiments), again after hypoglossal and recurrent laryngeal nerve section, and finally after induction of generalized skeletal muscle relaxation by administration of pancuronium (1 mg/kg iv).

The second series of experiments (n = 12) involved section of the ansa hypoglossi and SLN. Glossopharyngeal motor nerve responses to UA pressure and asphyxia were recorded first with all afferent pathways intact and then after section of the ansa hypoglossi (n = 6) or the SLN (n = 6), again after bilateral section of the remaining afferent pathway, once again after section of the hypoglossal and recurrent laryngeal nerve section, and finally after neuromuscular blockade.

When the hypoglossal nerve was divided to selectively paralyze tongue muscles, the main trunk of the hypoglossal nerve was cut in its midportion so that afferents traveling in the ansa hypoglossi were not damaged by the procedure. The recurrent laryngeal nerves were sectioned at the level of the tracheostomy. After neuromuscular blockade, the adequacy of anesthesia during paralysis was ensured by...
regular verification of no significant systemic arterial pressure or respiratory motor response to paw pinch.

Arterial blood gas samples (Table 1) were drawn intermittently to monitor arterial PO2, PCO2, and pH (Ciba Corning 278 blood gas system). Sodium bicarbonate (1 M) was administered intravenously as required to correct metabolic acidosis. At the end of each experiment, animals were killed by an overdose of pentobarbitone sodium (200 mg/kg iv).

Data analysis. Glossopharyngeal motor nerve activity was quantified in arbitrary units as the difference between the tonic end-expiratory activity and the peak activity reached during inspiration. Measurements were made on the control breath immediately before each intervention and during the first breath of UANP stimuli. The slope of the asphyxic response taken from the first to the last breath of a 20-s apnea was also determined and analyzed. ANOVA for repeated measures was used to test statistical hypotheses. Student-Newman-Keuls test was used for post hoc multiple comparisons. Data were logarithmically transformed to eliminate skewness or to homogenize variance across groups. \( P < 0.05 \) was accepted as indicating a statistically significant result.

RESULTS

Ansa hypoglossi section and glossopharyngeal motor activity. Ansa hypoglossi section reduced baseline glossopharyngeal activity and its responses to \(-20\text{-cmH}_2\text{O}\) UANP and asphyxia (Figs. 1B and 2B). Mean data from the 12 experiments in which the ansa hypoglossi were the first or only afferent pathway to be cut are shown in Fig. 3. UANP at \(-20\text{-cmH}_2\text{O}\) increased \( P < 0.001, n = 12 \), whereas \(+10\text{-cmH}_2\text{O}\) UA pressure suppressed, glossopharyngeal activity \( P < 0.001 \). Ansa hypoglossi section significantly reduced baseline glossopharyngeal activity (i.e., that recorded at \(0\text{-cmH}_2\text{O}\) UA transmural pressure, \( P < 0.001 \)). The responses to UA transmural pressure (Fig. 3A) and asphyxia (Fig. 3B) were also reduced \( P < 0.05 \) but were not abolished. This was true whether the response to asphyxia was expressed in terms of the increment in activity or the percent change in activity (latter data not shown).

Section of the ansa hypoglossi produced no significant change in respiratory timing \( P > 0.05 \); see Fig. 9A) or arterial blood gas composition (Table 1).

SLN section and glossopharyngeal motor activity. The SLN was the first or only afferent nerve to be cut in 12 experiments. With the SLN intact, UANP significantly increased and positive transmural pressure significantly decreased glossopharyngeal activity (see Fig. 6A; \( P < 0.001 \) for both stimuli). Section of the SLN significantly increased baseline glossopharyngeal discharge (Fig. 4B, see Fig. 6A; \( P < 0.001 \)) and its response to asphyxia (Figs. 5B and 6B; \( P < 0.05 \)). This was true whether the response to asphyxia was expressed in terms of the increment in activity or the percent change in activity (data not shown). SLN section abolished the response to changes in UA transmural pressure (Figs. 4B and 6A; \( P < 0.001 \)).

No significant change in respiratory timing \( P > 0.05 \); see Fig. 9B) or arterial blood gas composition (Table 1) occurred after SLN denervation.

Muscle relaxation and glossopharyngeal activity after afferent nerve section. Section of the hypoglossal and recurrent laryngeal nerves, relaxing tongue and laryngeal muscles, significantly reduced baseline glossopharyngeal activity, i.e., the activity at \(0\text{-cmH}_2\text{O}\) UA transmural pressure (Fig. 7; \( P < 0.01 \)). The reduction was significantly greater in the group of animals where prior SLN section had increased glossopharyngeal activity but was present whether the ansa hypoglossi (Fig. 7A; \( P < 0.01, n = 6 \)), the SLN (Fig. 7B; \( P < 0.001, n = 6 \)), or both (Fig. 7C; \( P < 0.01, n = 12 \)) had been cut (3-way ANOVA). Hypoglossal and recurrent laryngeal nerve section abolished the response to changes in UA transmural pressure (Figs. 1C, 4C, and 7; \( P < 0.01 \)) and significantly reduced the response to asphyxia (Fig. 8; \( P < 0.05 \)). ANOVA did not
detect any interaction between the effect of UA muscle relaxation on the response to asphyxia and which afferent pathways had been interrupted.

Subsequent neuromuscular blockade, paralyzing all remaining skeletal muscle, further reduced baseline glossopharyngeal activity (Figs. 1D, 4D, and 7; \( \text{P}/\text{H} \leq 0.001 \)) and its response to asphyxia (Figs. 2D, 5D, and 8; \( \text{P}/\text{H} \leq 0.01 \)) over and above the reductions caused by selective UA motor nerve denervation. UA motor activity and excitability were significantly reduced whether or not the ansa hypoglossi and/or the SLN had been interrupted (Fig. 7). Again, ANOVA did not detect an interaction between the impact of neuromuscular blockade and which afferent pathway(s) had been sectioned.

Respiratory timing (Fig. 9C) and arterial blood gas composition (Table 1) were not significantly affected (\( \text{P} > 0.05 \)) by selective denervation of the hypoglossal or recurrent laryngeal nerves or after neuromuscular blockade with pancuronium.

**DISCUSSION**

This study shows that glossopharyngeal activity and excitability were reduced by ansa hypoglossi section and were increased by section of the SLN. Muscle relaxation caused a further reduction in glossopharyngeal motor activity, which occurred independently of afferent fibers in the ansa hypoglossi or the SLN, as it occurred when both of these nerves had been sectioned. These findings, therefore, do not entirely support our initial hypothesis that muscle relaxation reduces UA motor discharge as a result of a change in activity in one or both of these afferent systems.

*Ansa hypoglossi afferents tonically excite UA motor control.* Section of the ansa hypoglossi reduced glossopharyngeal motor excitability, resulting in a reduction in baseline activity and responses to UA transmural pressure and brief asphyxia. These findings suggest that hypoglossal afferents tonically excite glossopharyngeal motor systems. This is a novel observation. Very little is known about the properties of hypoglossal afferents or the reflex responses evoked by their activation. They are stimulated by tongue stretch, muscle activity, and UANP (1, 11, 34). The latter stimulus is presumed to act by stretching the tongue (1). In the rat, hypoglossal afferents have been shown to originate from muscle spindles in the base of the tongue, albeit in very small numbers (20, 28). The majority of these afferents, especially in lower mammals, arise from simpler proprioceptive endings (4, 11).

Electrical stimulation of hypoglossal afferents excites laryngeal muscles (25, 35), inhibits masseter muscle activity (19), and causes excitatory-inhibitory responses in tongue muscles (10). There are no studies of the reflex UA or respiratory pump...
muscle responses to activation of tongue proprioceptors by stretch or vibration. Such experiments would be difficult to control, because a wide variety of deep and mucosal mechano-receptors in the tongue and linked UA structures would be affected by tongue stretch or vibration, including afferents arising from the larynx (36). However, the present finding, revealing a tonic excitatory input from hypoglossal afferents to UA motor systems, suggests that these afferents may have a role in regulating UA patency.

**SLN afferents tonically inhibit UA motor nerve activity.** SLN section increased baseline glossopharyngeal activity and its response to asphyxia but abolished the response to UA pressure changes. These findings reinforce prior observations from our laboratory (23) showing that SLN afferents tonically inhibit UA motor activity in rats. Although changes in respiratory pattern and ventilation have been reported after chronic SLN transection in sleeping dogs (2), rats (18), and neonatal guinea pigs (3), no other laboratory has reported a tonic influence of SLN afferent activity on UA motor outflow (6, 16, 30). This may be confined to the rat or may be particularly marked in this species.

The reflex glossopharyngeal response to changes in UA pressure was abolished after bilateral SLN section, confirming that, in the rat, receptors outside the larynx do not contribute to the response to UA transmural pressure (23). The rat differs from other species in this respect, because in the cat (6, 7), rabbit (14), dog (2), and human (5), nasal/nasopharyngeal receptors contribute to the reflex responses to UANP. The absence of an extralaryngeal contribution in the rat is not simply due to the pharynx collapsing under anesthesia so that pharyngeal receptor endings are not exposed to changes in transmural pressure. The pressure stimulus can be detected by an intraluminal catheter throughout the entire length of the UA under the same conditions used in these experiments (23).

Muscle relaxation and UA motor control after afferent nerve section. Glossopharyngeal activity and its responses to excitatory inputs, such as asphyxia, were suppressed when tongue and laryngeal muscles were paralyzed. Despite this decrease, the additional relaxation of all other skeletal muscles by neuromuscular blockade caused further reductions in glossopharyngeal activity and excitability. Failure of the response to asphyxia during paralysis shows that the excitability of UA motor (or premotor) neurons was markedly reduced, despite excitation from chemoreceptors, which is normally a potent stimulus to UA motor systems. A breakthrough excitation of glossopharyngeal motor nerve activity occurred toward the end of the period of exposure to asphyxia in some animals, confirming that the reflex response to asphyxia was present but

**Fig. 5.** Effect of SLN and ansa hypoglossi section on glossopharyngeal motor responses to asphyxia in anesthetized rat. Original records show effect of asphyxia (withholding mechanical ventilation for 20 s) on glossopharyngeal nerve activity in intact state (A), after bilateral SLN section (B), after subsequent bilateral ansa hypoglossi section (C), and after neuromuscular blockade with pancuronium (D).
greatly reduced (Fig. 2). Reduced excitability appears to be confined to UA motor systems, because marked recruitment of phrenic motor drive by asphyxia was consistently observed in all animals, even with paralysis.

Our initial hypothesis was that muscle relaxation affects the control of UA motor systems by altering the discharge of some group or groups of afferent fibers, namely, those in the ansa hypoglossi and SLN, and that loss of feedback from these...
afferent inputs reduces the excitability of UA motor or premotor neurons. However, muscle relaxation further reduced excitability, even when one or both of the afferent pathways under study had been interrupted. This finding refutes our hypothesis that one or both of these pathways is exclusively responsible for the changes that occur with muscle relaxation. It is important to note that it does not completely rule out involvement of these afferent pathways in mediating some of the changes that occur with muscle relaxation. These experiments, however, did not compare the effects of muscle relax-
ation before and after ablation of the afferent fibers. It is possible that the response to muscle relaxation was altered or reduced by ansa hypoglossal or SLN section, a phenomenon that would not be detected in the experiments reported here. Nonetheless, part or all of the response to muscle relaxation appears to be mediated via afferent pathways other than the ansa hypoglossi or the SLN.

A number of other afferent systems could be responsible, but it is only possible to speculate regarding their involvement. For example, the lingual nerve conducts afferent fibers from tongue mechanoreceptors, which are sensitive to local deformation but are also stimulated by tongue stretch and muscle contraction (21). These afferents are known to influence hypoglossal motoneurons (22). There are also afferent fibers from the tongue in the trigeminal and glossopharyngeal nerves (32). Trigeminal afferents from mechanoreceptors in the nasal airway, commonly found in branches of the trigeminal nerve innervating the posterior nose, nasopharynx, and palate (26, 31), are also influenced by muscle activity. Furthermore, trigeminal afferents from the temporomandibular joint and masticatory muscles greatly influence hypoglossal motor activity (8, 13, 17, 29). The glossopharyngeal nerve itself, although intact on only one side in these experiments, contains afferent fibers that may be influenced by muscle activity (7, 33).

Although localized paralysis of tongue and laryngeal muscles reduced glossopharyngeal activity, neuromuscular blockade caused a further reduction in excitability. This could be due to relaxation of other UA muscles, further altering afferent input from UA mechanoreceptors, to relaxation of craniofacial muscles, changing the discharge of associated muscle and joint proprioceptors, or perhaps even to alterations in the activity of more remote proprioceptors, such as chest wall afferents.

An important limitation of the present work is that the activity of only one UA motor nerve was examined. Muscle relaxation has been shown to suppress the activity of other motor outflows (9, 24). However, the present observations on the glossopharyngeal motor nerve should be generalized cautiously. Most importantly, the influence of ansa hypoglossi afferents on other UA motor systems, especially the hypoglossal motor system, should be examined. Furthermore, although muscle relaxation may reduce the activity of all UA motor outflows, the relative role of changes in individual UA afferent activity, during muscle relaxation, in mediating this reduction in UA motor nerve activity may vary from one motoneuron pool to another. Finally, we do not know whether the decrease in UA motor drive after muscle relaxation is due to a withdrawal of respiratory-related drive, tonic postural drive, or both. Paralysis did not affect motor drive to the diaphragm. This may be analogous to the situation during normal sleep, in which excitability of UA and postural muscles is reduced without any concomitant change in motor drive to respiratory-related pump muscles. We did not examine changes in drive to postural muscles after neuromuscular blockade and are not aware of any previous studies in this area.

In conclusion, ansa hypoglossi afferents tonically excite, whereas SLN afferents tonically inhibit, glossopharyngeal activity. Reduced motor drive to the UA by muscle relaxation survives bilateral interruption of ansa hypoglossi and SLN afferents, indicating that some or all of the effect of muscle relaxation is mediated by alterations in the activity of afferent fibers other than those in the ansa hypoglossi or SLN.

GRANTS

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REFERENCES