Upper airway muscle paralysis reduces reflex upper airway motor response to negative transmural pressure in rat

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Ryan, Stephen, Walter T. McNicholas, Ronan G. O’Regan, and Philip Nolan. Upper airway muscle paralysis reduces reflex upper airway motor response to negative transmural pressure in rat. J Appl Physiol 94: 1307–1316, 2003—The reflex upper airway (UA) motor response to UA negative pressure (UANP) is attenuated by neuromuscular blockade. We hypothesized that this is due to a reduction in the sensitivity of laryngeal mechanoreceptors to changes in UA pressure. We examined the effect of neuromuscular blockade on hypoglossal motor responses to UANP and to asphyxia in 15 anesthetized, thoracotomized, artificially ventilated rats. The activity of laryngeal mechanoreceptors is influenced by contractions of laryngeal and tongue muscles, so we studied the effect of selective denervation of these muscle groups on the UA motor response to UANP and to asphyxia, recording from the pharyngeal branch of the glossopharyngeal nerve (n = 11). We also examined the effect of tongue and laryngeal muscle denervation on superior laryngeal nerve (SLN) afferent activity at different airway transmural pressures (n = 6). Neuromuscular blockade and denervation of laryngeal and tongue muscles significantly reduced baseline UA motor nerve activity (P < 0.05), caused a small but significant attenuation of the motor response to asphyxia, and markedly attenuated the response to UANP. Motor denervation of tongue and laryngeal muscles significantly decreased SLN afferent activity and altered the response to UANP. We conclude that skeletal muscle relaxation reduces the reflex UA motor response to UANP, and this may be due to a reduction in the excitability of UA motor systems as well as a decrease of the response of SLN afferents to UANP.

UA motor activity in the anesthetized rat (26), and that this response is dependent on laryngeal mechanoreceptors, because it is abolished by bilateral section of the superior laryngeal nerves (SLNs).

Janckzewski (10) has shown that the reflex hypoglossal (XII) motor response to UANP is attenuated by neuromuscular blockade in decerebrate or anesthetized rabbits. The mechanism by which muscle paralysis reduces the reflex response to subatmospheric pressure in the UA is not known. It is reasonable to suggest that muscle relaxation might reduce the sensitivity of airway mechanoreceptors to changes in transmural pressure. It is known that the discharge of many pressure-sensitive airway mechanoreceptors is influenced by the activity of nearby skeletal muscles (27–29, 31, 35). It has also been proposed (10) that loss of muscle tension might somehow alter the excitability of UA motoneurons, perhaps by reducing a tonic excitatory input from muscle spindles (32), proprioceptors (10), or other mechanoreceptors whose activity is modulated by skeletal muscle tension.

We formally approached this question by hypothesizing that the effect of muscle relaxation on the reflex response to UANP is entirely due to a reduction in the sensitivity of laryngeal mechanoreceptors to changes in airway transmural pressure. This hypothesis has three specific predictions, which are tested in the experiments reported here.

First, if muscle relaxation attenuates the reflex response to UANP because of its effect on laryngeal mechanoreceptors, then paralysis should not alter the UA motor response to an unrelated excitatory stimulus such as asphyxia. We tested this prediction by recording the increase in XII motor activity evoked by UANP and comparing this with the XII response to a brief episode of asphyxia, before and after neuromuscular blockade in chloralose-anesthetized, thoracotomized, artificially ventilated rats. The neuromuscular blockers, atracurium and pancuronium, employed in this study do not penetrate into the cerebrospinal fluid unless the blood-brain barrier has been compromised (4, 36). We conducted experiments using these neuro-
muscular blockers with different pharmacodynamic properties (5, 11, 14, 23, 33) to reduce the possibility of spurious results attributed to the pharmacology of any one particular agent.

Second, if muscle relaxation reduces the response to UANP because laryngeal mechanoreceptors are less sensitive to changes in pressure, then selectively paralyzing only those muscles that influence laryngeal mechanoreceptor discharge should have the same effect as generalized muscle paralysis induced by neuromuscular blockade. Afferent activity in the rat SLN is influenced not only by contractions of intrinsic laryngeal muscles innervated by the recurrent laryngeal nerve (RLN) but also by the activity of tongue and hyoid muscles supplied by the XII nerve (31). We examined the effect of bilateral section of these motor nerves on the reflex UA motor response to UANP and to asphyxia. Given that these experiments required us to section the XII nerve in the course of the protocol, we made our recordings from the pharyngeal branch of the glossopharyngeal (Ph-IX) nerve. We studied this motor outflow because its responses should be comparable to those of the XII nerve. The Ph-IX nerve primarily innervates the stylopharyngeus muscle, an airway dilator, but also supplies palatal and pharyngeal constrictor muscles via the pharyngeal plexus (13). The mechanical effect of activating the Ph-IX nerve is broadly similar to that of stimulating the XII nerve (13), and we have previously reported that it is activated by UANP in a similar fashion to the XII nerve (26).

Third, if our hypothesis is correct, then muscle relaxation will diminish the SLN afferent response to UANP, to an extent that is commensurate with the reduction of the reflex response to this stimulus. We tested this by examining the effect of bilateral XII nerve and RLN section on afferent activity of the SLN at a range of airway transmural pressures.

We conducted an additional series of experiments to eliminate a possible artifactual explanation for the effect of muscle paralysis on the reflex response to UANP. Paralysis might simply cause sections of the pharynx to collapse, so the pressure stimulus is not faithfully transmitted to, or affects only limited regions of, the airway (3). We directly measured the airway transmural pressure at a number of points along the length of the airway before and after muscle relaxation to determine whether this was the case.

METHODS

General procedures. Experiments were conducted in 38 adult male Sprague-Dawley rats. The work was performed under license from the Department of Health and Children, Ireland, and conformed to national and university guidelines regarding animal experimentation. Surgical anesthesia was induced with pentobarbitone sodium (60 mg/kg ip; Sagatal, Rhone Merieux, Dublin, Ireland) and maintained with α-chloralose (10 mg/kg iv; Sigma Aldrich, Dorset, UK) administered as required 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 (Harvard Instruments, Kent, UK) to maintain rectal temperature close to 37°C. The right femoral artery and vein were cannulated to record systemic arterial pressure (model P23Dd, Statham, Heto Rey, PR) and for injection of drugs, respectively.

Animals were tracheostomized, with care taken to preserve the RLNs, bilaterally thoracotomized, and artificially ventilated (CWE SAR-830/P, Linton Instrumentation, Norfolk, UK; inspired O2 fraction = 0.33, respiratory rate = 90 breaths/min, inspiratory time = 300 ms, tidal volume = 1–2 ml, positive end-expiratory pressure = 1–2 cmH2O). A differential pressure transducer within the ventilator continuously monitored tracheal pressure.

UA preparation. Atropine sulfate (0.01 mg/kg iv; Antigen Pharmaceuticals, Roscrea, Ireland) was administered to reduce UA secretions. The UA was exposed to changes in transmural pressure as previously described (21). In short, the UA was isolated by inserting a second cannula 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 by sealing with vaseline (Fig. 1). The laryngeal cannula and mask were connected together and then to a pressure reservoir via a

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Fig. 1. Schematic diagram of rat upper airway preparation.
solenoid valve. The pressure reservoir could be evacuated or pressurized to a predetermined level in the range from +10 to −30 cmH2O so that activation of the solenoid valve allowed controlled application of that pressure to the isolated UA segment. Pressures were applied simultaneously to the mask and the laryngeal cannula for 5 s. Airflow through the UA during a change in transmural pressure indicated a leak, which tended to cause pressure gradients along the length of the airway and thus glottic or pharyngeal occlusion. Flow was detected by using a pneumotachograph attached to the laryngeal cannula (Fig. 1; Fleisch 00, Linton Instrumentation, and DP45 ±2 cmH2O, Validyne, Northridge, CA). If flow through the UA was detected, these trials were excluded from analysis and the leak eliminated.

A transducer attached to the laryngeal cannula monitored the pressure applied to the UA in all experiments (Fig. 1; DP45 ±5 cmH2O, Validyne). Pressure within the lumen of the UA was directly measured in six animals by using a saline-filled catheter and pressure transducer (model P23Dd, Statham). The cannula was passed through the laryngeal cannula until its tip appeared through one nostril. The catheter was withdrawn in steps of 5 mm, and the pressure was recorded at each point when −20 cmH2O was applied.

The effect of UANP on UA motor discharge was compared with the effect of brief asphyxia, induced by a 5-s apnea, where the ventilator was stopped and the lungs deflated in these thoracotomized animals to the volume determined by the applied positive end-expiratory pressure.

**Nerve recording.** All neural recordings were obtained by using glass suction electrodes filled with phosphate-buffered saline solution (0.01 M; Sigma Aldrich). Electrodes were pulled by using a Narshige electrode puller (PC-10), broken back until the bore diameter matched that of the cut end of the nerve (20–80 μm depending on the nerve) and fire polished (Intracel Microforge). Activity was amplified (Neurolog NL100AK preamplifier and NL104 amplifier, Digitimer, Welwyn Garden City, UK), filtered (0.3–2 kHz, NL 125), processed by a leaky integrator (time constant = 100 ms, NL 703), and fed to an audiomonitor and an oscilloscope.

The neural recordings made in any given experiment depended on the experimental protocol. Efferent activity from the right phrenic nerve was recorded in all animals. In 15 animals, the right XII nerve was carefully dissected, and motor activity was recorded from the cut central end of the main trunk. In a separate series of experiments (n = 11), motor activity from the Ph-IX nerve was recorded. The carotid sinus nerve was preserved by sectioning the Ph-IX nerve distal to the point where these nerves converge. In a further six animals, neural recordings of whole nerve afferent activity were made from the peripheral end of the cut right SLN, distal to its communication with the aortic nerve.

Given that these experiments were designed to examine the effect of skeletal muscle relaxation on the activity of UA motor nerves, it was important to ensure that the neural recordings were not contaminated by electrical activity of nearby skeletal muscles. We conducted supplementary experiments in two rats that showed that all activity recorded from the distal cut end of the XII nerve was abolished by crushing the nerve proximal to the recording site.

Neural signals, together with systemic arterial pressure, tracheal pressure, UA pressure, and UA airflow, were recorded and stored on a computer using a CED micro1401 interface and Spike 2 software (CED, Cambridge, UK).

**Experimental protocols.** Four series of experiments were conducted. Series 1 examined the XII motor response to UANP (−10 to −30 cmH2O) and brief asphyxia (5-s apnea) before and after neuromuscular blockade with atracurium (n = 8) or pancuronium (n = 7). Atracurium was administered as a bolus dose of 4 mg/kg iv followed by continuous infusion of 12 mg·kg−1·h−1 iv. Pancuronium was given as a bolus dose of 1 mg/kg iv and an infusion thereafter of 2 mg·kg−1·h−1 iv. The adequacy of anesthesia during paralysis was ensured by regularly checking that there was no significant systemic arterial pressure or respiratory motor response to paw pinch.

Series 2 (n = 11) investigated the effect of denervating tongue, hyoid, and laryngeal muscles on the Ph-IX motor response to UANP and asphyxia. The XII nerve and RLN were identified bilaterally and marked. The Ph-IX response to UANP of −10 to −30 cmH2O and 5 s of asphyxia was recorded, and either the XII nerve or the RLN was then cut on both sides. The choice of nerve was randomized, and in five cases the RLNs were cut first, whereas in six animals the XII nerves were sectioned first. The stimulus-response relationship to UANP was repeated, and the effect of asphyxia was recorded. The remaining motor nerve was cut bilaterally, and the response to UANP and to asphyxia was recorded again.

Series 3 examined (n = 6) the whole nerve afferent activity recorded from right SLN at UA transmural pressures from +10 to −30 cmH2O and before and bilateral section of the RLN and XII nerve.

Series 4 (n = 6) recorded the pressure detected at points along the UA during −20-cmH2O stimuli, first in the unparalyzed state, then after bilateral section of the RLN and XII nerves, and again after neuromuscular blockade with atracurium.

Arterial blood-gas samples were drawn intermittently to monitor arterial Po2, arterial Pco2, and arterial pH levels (model 278 blood-gas system, Ciba Corning). Sodium bicarbonate (1 M iv) was administered as required to correct metabolic acidosis. At the end of each experiment animals were killed by an overdose of pentobarbital sodium (200 mg/kg iv).

**Data analysis.** The phasic inspiratory activity of UA motor nerves was quantified in arbitrary units as the difference between the tonic end-expiratory and the peak activity reached during inspiration, during the control breath immediately before each intervention, during the first breath of UANP stimuli, and during the last breath of each 5-s period of asphyxia. Afferent discharge in the whole SLN was quantified by recording the level of activity above electrical zero at end inspiration and end expiration. The latter value was taken to be tonic activity, and difference between these values as the degree of inspiratory modulation of this activity.

ANOVA for repeated measures was used to test statistical hypotheses. The Student-Newman-Keuls test was used for post hoc multiple comparisons. Data were log transformed to eliminate skewness or to homogenize variance across groups. P < 0.05 was accepted as indicating a statistically significant effect.

**RESULTS**

Effect of neuromuscular blockade on XII motor response to UANP and brief asphyxia. Preparalysis phasic XII motor nerve activity significantly increased (P < 0.0001; Figs. 2 and 3) in response to all UANP stimuli. Neuromuscular blockade induced with either atracurium (Figs. 2 and 3A) or pancuronium (Fig. 3B) significantly reduced baseline phasic XII nerve activity (that recorded at 0-cmH2O UA transmural pressure; P = 0.01; n = 15, Fig. 3) and markedly decreased the
reflex response to UANP ($P < 0.0001$; Fig. 3). Although small residual responses to $-20$- and $-30$-cmH$_2$O UANP remained after atracurium (Fig. 3A), no significant effect of UANP on XII motor nerve activity was detected after muscle relaxation with pancuronium (Fig. 3B).

Asphyxia significantly increased XII nerve activity ($P < 0.01$; Fig. 3) before and after neuromuscular blockade. The magnitude of this response was significantly reduced after neuromuscular blockade with atracurium (Fig. 3A) but was not statistically different after paralysis with pancuronium (Fig. 3B).

**Effect of XII nerve and RLN section on Ph-IX motor response to UANP and brief asphyxia.** We first compared responses in the intact state with responses after bilateral section of both the XII nerve and the RLN across all 11 animals in this experimental group (Fig. 4). This caused a small but significant reduction in baseline Ph-IX nerve activity ($P < 0.05$; $n = 11$, Fig. 4), greatly reduced the responses to UANP ($P < 0.0001$), and moderately attenuated the response to asphyxia ($P < 0.001$).

We then examined the effect of bilateral section of either the XII nerve or the RLN alone. Bilateral section of the XII nerve alone abolished the Ph-IX motor response to UANP ($P < 0.0001$; $n = 6$, Fig. 5A) and caused a small, significant attenuation of the response to asphyxia ($P = 0.0004$; Fig. 5A). Subsequent section of the RLN had no significant additional effect. However, bilateral section of the RLN alone, with intact XII nerve, caused a moderate reduction in the response to UANP ($P < 0.001$; $n = 5$, Fig. 5B) with significant responses remaining at pressures of $-20$ and $-30$ cmH$_2$O ($P < 0.02$; Fig. 5B). These responses were abolished by subsequent bilateral section of the XII nerve ($P < 0.001$). The effect of asphyxia was unaltered by bilateral RLN section alone (Fig. 5B). Although the animals were randomly assigned during the series to

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**Fig. 2.** Effect of neuromuscular blockade with atracurium on the hypoglossal (XII) motor response to upper airway negative pressure. Traces show effect of $-20$-cmH$_2$O upper airway negative pressure on XII and phrenic motor nerve activity before (A) and after (B) neuromuscular blockade with atracurium in an anesthetized, thoraetomized, mechanically ventilated rat.

**Fig. 3.** Effect of neuromuscular blockade on the XII motor response to upper airway negative pressure. Shown are group responses to upper airway negative pressure on the first breath of exposure to pressure and brief asphyxia (5-s cessation of artificial ventilation) before (solid bars) and after (open bars) paralysis induced with atracurium (A; $n = 8$ rats) and pancuronium (B; $n = 7$ rats). Values are means ± SE. Con, 0 cmH$_2$O; $-10$, $-10$ cmH$_2$O; $-20$, $-20$ cmH$_2$O; $-30$, $-30$ cmH$_2$O; au, arbitrary units. *Significant effect of the applied pressure compared with 0 cmH$_2$O, $P < 0.05$. †Significant effect of neuromuscular blockade, $P < 0.05$. 

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**Fig. 4.** Effect of bilateral section of both the XII nerve and the RLN on Ph-IX motor response to UANP. This caused a small but significant reduction in baseline Ph-IX nerve activity ($P < 0.05$; $n = 11$, Fig. 4), greatly reduced the responses to UANP ($P < 0.0001$), and moderately attenuated the response to asphyxia ($P < 0.001$).
have either the XII nerve or the RLN sectioned first, the baseline Ph-IX motor discharge and responses to UANP and asphyxia were statistically significantly greater in the experimental subgroup where the RLN was the first to be cut.

Although bilateral section of the XII nerve and RLN clearly reduced basal Ph-IX motor discharge when all 11 animals were considered together (Fig. 4), there was no significant effect of cutting the XII nerve or the RLN alone, probably due to the reduced statistical power when considering the two smaller subgroups of animals.

**Effect of XII nerve and RLN section on SLN afferent responses to upper airway transmural pressure.** The peripheral cut end of the right SLN was tonically active in all six animals studied. This tonic discharge fluctuated slightly with respiration in five cases, decreasing during neural inspiration in four (Fig. 6A) and increasing in one. UANP caused a reduction in tonic SLN activity (Figs. 6 and 7A), associated with an increase in phasic inspiratory afferent discharge (P < 0.03; Figs. 6A and 7C). Positive UA pressures evoked an immediate increase in tonic SLN discharge that was not modulated by respiration.

Neither baseline right SLN afferent activity, nor its response to negative pressure, nor the marked respiratory modulation of afferent activity seen during negative pressure stimuli significantly changed after section of the contralateral SLN (data not shown).

Bilateral section of both the XII nerve and the RLN significantly reduced baseline tonic SLN afferent activity (P < 0.001; Figs. 6 and 7A) and caused a shift to the left of the relationship between transmural pressure and afferent discharge. SLN afferent activity still decreased in response to UANP, and the absolute level of SLN activity reached during negative pressure stimuli of −10, −20, and −30 cmH2O was similar in both the unparalyzed and paralyzed states (P > 0.6; Figs. 6 and 7A). However, the change in end-expiratory afferent activity in response to changes in transmural pressure was significantly reduced at all levels of subatmospheric pressure below −5 cmH2O and to positive pressure stimuli of +10 cmH2O (P < 0.05; Fig. 7B).

The respiratory modulation of SLN afferent activity at baseline and during negative pressure stimuli was abolished by bilateral section of the XII nerve and the RLN (P < 0.0001; Fig. 6B).

**Direct measurement of UA transmural pressure before and after paralysis.** Figure 8 shows the pressure measured at different points along the airway when...
−20 cmH₂O UANP was applied simultaneously to both the face mask and upper tracheal cannula, as it was for all experiments reported above. The applied pressure was detected at all points along the length of the airway, although the stimulus was somewhat attenuated in a region 25–45 mm from the external nares. Bilateral section of the XII nerve and the RLN, and subsequent administration of the neuromuscular paralysis and the upper airway pressure reflex.

Fig. 6. Effect of local laryngeal muscle paralysis on superior laryngeal nerve (SLN) afferent response to upper airway negative pressure. Original record demonstrates the response of the whole right SLN afferent activity to −20 cmH₂O before (A) and after (B) local laryngeal muscle paralysis was induced by bilateral section of the XII and the recurrent laryngeal nerves. Phrenic motor activity is also shown.

Fig. 7. Group effect of local laryngeal muscle paralysis on SLN afferent activity. Values are means ± SE; n = 6 rats. Graphs show the effect of upper airway transmural pressure on the absolute level of tonic expiratory SLN afferent activity (A) and the change in expiratory SLN afferent activity from baseline (B) before (●) and after (□) bilateral section of both the XII and recurrent laryngeal nerves. C: phasic SLN afferent activity in the intact state.

*Significant effect of the applied pressure compared with 0 cmH₂O, P < 0.05. †Significant effect of sectioning both XII and recurrent laryngeal nerves, P < 0.05.
alyzing agent atracurium, did not significantly alter the pressure recorded within the UA.

DISCUSSION

We hypothesized that muscle relaxation reduces the reflex response to UANP solely because of a reduction in the sensitivity of laryngeal mechanoreceptors to changes in transmural pressure. However, our data are not consistent with this hypothesis, and they strongly suggest that muscle relaxation, by some as yet unclear mechanism, reduces the excitability of UA motor systems. Although this may contribute to the diminished reflex response to UANP, the change in excitability is relatively small. We conclude, therefore, that the effect of muscle relaxation on the reflex response to UANP is most likely due to a combination of reduced sensitivity of laryngeal mechanoreceptors to changes in airway transmural pressure and reduced excitability of UA motor or premotor circuits.

Effect of neuromuscular blockade on XII motor response to UANP and brief asphyxia. Our experiments that used neuromuscular blockade support and extend the findings of Janckzewski (10), who reported that muscle relaxation with vecuronium reduced the reflex response to UANP in the rabbit. We confirm that this reflex is also diminished after neuromuscular blockade in the rat, although the magnitude of the effect is greater, with the response almost completely abolished in the rat, in contrast with the moderate reduction reported for the rabbit. This may be a genuine species difference, or it may be attributable to the different neuromuscular blocking agents used or some other disparity in experimental conditions.

The data indicate, contrary to our original hypothesis, that the XII motor system is less excitable in the paralyzed state. First, resting phasic inspiratory XII activity was less after muscle relaxation, an effect also noted by Janckzewski (10). Second, the XII response to asphyxia was diminished, although this effect was observed only after paralysis with atracurium and not with pancuronium. The most likely explanation for this discrepancy is that there were differences in the degree or distribution of muscle relaxation caused by these agents at the doses employed in this study. We did not conduct the formal dose-effect experiments required to establish whether there is a real difference between these drugs in terms of their effect on the asphyxic response.

The moderate reduction in basal XII nerve activity and its response to asphyxia contrast sharply with the very marked depression or complete extinction of the UANP response. This suggests that diminished excitability of UA motor or premotor circuits accounts for some but not all of the effect of muscle relaxation on the UANP reflex. However, it is important to note that the UANP and asphyxic responses were not well matched in the unparalyzed state, in that the magnitude of the UA motor responses to these stimuli was different, so quantitative comparisons of the effects of paralysis on these different responses should be made with caution.

Effect of XII nerve and RLN section on Ph-IX motor response to UANP and asphyxia. Denervation of tongue, hyoid, and laryngeal muscles, with the objective of selectively paralyzing only those muscles known to affect laryngeal mechanoreceptors (18, 31), had effects on UA motor control that were strikingly similar to those of neuromuscular blockade. The ongoing phasic inspiratory activity of the Ph-IX nerve was reduced, its recruitment by asphyxia was decreased, and its response to UANP was greatly diminished. The data suggest that relaxation of muscles of the tongue and hyoid apparatus innervated by the XII nerve play the major role in this effect. RLN section caused some attenuation of the Ph-IX response to UANP, but it did not alter the effect of asphyxia, whereas XII nerve section had a more marked suppressant effect on the UANP reflex and also significantly reduced the response to asphyxia. These findings confirm that muscle relaxation decreases the excitability of UA motor circuits, as reflected by the reduced Ph-IX activity at rest and in asphyxia. Importantly, this phenomenon is demonstrated without the use of neuromuscular blocking agents, excluding a central effect of these drugs or their metabolites as a possible mechanism. We also note that the effect of denervation on the reflex motor response to UANP was greater than the effect on activity at rest or during asphyxia. Moreover, in the case of RLN section, these effects were dissociated, so the UANP reflex was significantly reduced without an effect on the response to asphyxia. These disparities strongly support the idea, already suggested by the experiments involving neuromuscular blockade, that, although muscle relaxation reduces the excitability of UA motor systems, this alone is not sufficient to account for the much greater reduction in the reflex response to UANP.
This study did not anticipate an effect of paralysis on the excitability of UA motor systems, nor were the experiments designed to establish the mechanism of such an effect. We presume that muscle relaxation alters the activity of proprioceptors or other mechanoreceptors that have tonic inputs to the control of UA muscles and thus decrease tonic excitatory and/or increase tonic inhibitory influences on UA motoneurons. We can only speculate on which group or groups of sensory receptors might be responsible. The fact that section of the XII nerve alone is sufficient to alter UA excitability suggests that receptors within the UA itself play a role. The rat XII nerve contains afferent fibers from a very small number of muscle spindles and a much larger population of less specialized, low-threshold mechanoreceptors (2, 15, 24, 30, 40, 41). The lingual nerve also conducts afferent fibers from tongue mechanoreceptors, which are most sensitive to local deformation, but are also stimulated by tongue stretch and muscle contraction (25). The extensive trigeminal sensory innervation of the nose, palate, and nasopharynx includes a significant population of mechanoreceptors influenced by local muscle activity (29). Furthermore, if muscle relaxation alters jaw posture, this could also elicit trigeminal reflexes from temporomandibular joint receptors (17) or proprioceptors in the masticatory muscles (9, 34). The glossophageal nerve also includes many sensory fibers influenced by pharyngeal muscle activity and the conformation of the pharyngeal airway (7, 8, 16, 21, 39).

One important possibility is that muscle relaxation might render UA motor systems less excitable by modifying a tonic input from afferent fibers in the SLN. This input might arise from the same population of afferents that mediate the reflex response to UANP or from a different and functionally distinct group of mechanoreceptors. This idea is supported by our prior report that SLN afferents have a net tonic inhibitory influence on UA motor outflow, in that the discharge of a number of UA motor nerves is increased after bilateral section of the SLN (26). However, as discussed below, our recordings from SLN afferents, although not conclusive, are not consistent with this proposal.

**Effect of XII nerve and RLN denervation on SLN afferent activity.** The relaxation of UA muscles by sectioning of the XII nerve and the RLN altered SLN afferent activity and its response to changes in UA transmural pressure. The tonic afferent activity evident at zero transmural pressure was reduced by motor denervation. As a consequence, the change in afferent activity when transmural pressure became negative was also reduced, although the absolute level of afferent discharge remained unchanged. The marked phasic inspiratory bursts of afferent discharge that appeared when transmural pressure was made negative were eliminated by local muscle relaxation. The significance of these observations is not clear, principally because there is little quantitative information on how laryngeal afferent input is interpreted by the brain stem. It is not known whether the reflex response to UANP is evoked by the change in SLN sensory discharge, in which case the afferent signal is reduced by muscle relaxation, or whether the absolute level of SLN activity influences UA motor activity, in which case the afferent signal is unchanged. Furthermore, if the pattern of SLN afferent activity is of importance, and the phasic bursts of afferent activity during the negative pressure stimulus contribute to the evoked reflex response, then the afferent signal is radically altered by loss of muscle tone.

We proposed that muscle relaxation might decrease the excitability of UA motor systems by altering a tonic input from laryngeal afferents. The data presented here do not support this proposition. Local muscle denervation was associated with a decrease in ongoing SLN afferent activity. Our previous work indicates that the SLN has a net tonic inhibitory effect on UA motor outflow (26), so it would be expected that a reduction in tonic SLN afferent activity would diminish rather than suppress UA motor activity. However, this may well be too simplistic an analysis of our findings. The SLN contains a number of different groups of mechanosensitive afferents (29) with widely different functions. We cannot discern from recordings of mass afferent discharge how these different subpopulations of SLN afferents are affected by local muscle denervation. It could be argued that, although cutting the SLN and eliminating all of its afferent input has a net disinhibitory effect, a selective reduction in the activity of some afferent fibers secondary to muscle relaxation could still have a net inhibitory effect on UA motor discharge. It is a major limitation of the present study that we did not conduct single-unit recordings of laryngeal afferents to determine in detail how this diverse population is affected by loss of muscle tone.

The origin of the marked respiratory modulation of SLN afferent activity at negative transmural pressures is not clear. The observation is at variance with the finding of Sekizawa and Tsubone (31), who found, however, that subjecting the isolated UA to subatmospheric pressure reduced inspiratory modulation of SLN afferents. The modulation is most likely due to phasic contraction of pharyngeal muscles, given that it is abolished by local muscle paralysis. It is difficult to explain why it appears only when the airway is distorted by negative transmural pressure. We know it is not due to a reflex increase in UA muscle activity in response to UANP, because the pattern of afferent discharge is unaffected by cutting the contralateral SLN, which would abolish this reflex response. It is also unlikely that laryngeal receptors stimulated by UANP and by local muscle activity are responsible, because these receptors are very rare in the rat (31). The most probable explanation is that the respiratory modulation of these fibers by local muscle activity is altered when the airway is distorted by negative transmural pressure. We speculate that, at zero transmural pressure, local muscle activity tends to unload laryngeal mechanoreceptors, resulting in a small reduction in whole nerve afferent activity during inspiration. However, large changes in conformation of the airway wall that occur with application of negative transmural
pressure could alter the manner in which local muscle activity is coupled to the mechanoreceptor. Inspiratory contraction of the pharyngeal muscles might now load or stretch the receptor so that afferent activity increases during inspiration. This hypothesis remains to be tested by recording from single fibers of the SLN.

**Direct measurement of UA transmural pressure before and after paralysis.** We considered the possibility that paralysis may have caused large sections of the pharynx to collapse, so receptor fields normally affected by the distorting effects of UANP might no longer be exposed or not be responsive to UANP. The elimination of reflex responses to UANP by this artificial mechanism has been clearly demonstrated in the past (3). We ruled this out by showing that, under our experimental conditions, pressure could be detected at all points along the length of the UA before and after paralysis, although it was somewhat attenuated in the collapsible segment of the pharynx. It should be noted that our measurements do not eliminate the possibility that there was some narrowing or short regions of collapse within the UA.

**Implications.** This study has a number of important physiological and pathophysiological implications. We have clearly demonstrated that muscle relaxation has important effects on UA motor control, so UA motor circuits are less excitable, and the reflex response to negative transmural pressure is impaired. The findings have important implications for our understanding of the mechanisms maintaining pharyngeal patency during sleep. It is well known that sleep is associated with a fall in UA motor activity and a reduction in the reflex response to UANP (6, 37, 38). This is usually attributed to a disfacilitation of UA motor systems, due to withdrawal of a central excitatory drive to UA motoneurons (12). The present study indicates that a peripheral mechanism may contribute to these changes in UA motor control, in that changes in afferent feedback due to the loss of muscle tone associated with the sleeping state may affect both the excitability of UA motor circuits and the afferent signals reporting airway transmural pressure.

We allude to the possibility of species differences in the sensitivity of UA motor systems to the loss of muscle tone. If there are genuine differences between species, they may relate to differences in airway structure, so animals with a more linear airway, such as the rat, may be more susceptible to the effects of muscle relaxation compared with those animals, including rabbits and humans, in which the airway is more L shaped. It has clearly been shown that the anatomic conformation of the airway has a major influence on its neural control (22). The importance of alterations in peripheral feedback due to muscle relaxation in the control of the UA in humans remains to be determined.

There are a number of other important questions that deserve further investigation. First, the afferent pathway(s) responsible for the effect of reduced muscle tone on UA motoneuron excitability is not known. Second, we do not know whether muscle relaxation affects excitability in a uniform way across all UA motoneuron pools. We demonstrated an effect on two motor outflows, the XII and Ph-IX nerves. We did not conduct experiments to determine whether paralysis had a greater or lesser effect on functionally different motor systems, such as tongue protruder motor activity in the median branch of the XII nerve compared with tongue retractor activity in the lateral XII nerve, or on motor outflow to pharyngeal constrictor muscles in the pharyngeal branch of the vagus nerve.

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**REFERENCES**


