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The morphological and functional characteristics of the innervation of the subglottic mucosa of the larynx

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Summary

Neurohistological studies of the subglottic mucosa of the larynx in the cat have revealed the presence therein of nerve endings of varying morphology and distribution. Multichannel electromyographic studies of the effects of afferent discharges from the subglottic mucosal mechanoreceptors on the motor-unit activity of the laryngeal muscles indicated that these receptors exert significant reflexogenic effects on the activity of the laryngeal musculature during quiet breathing. The more important contribution of the subglottic mucosal mechanoreceptor reflexes to laryngeal muscle control during phonation is considered.

Introduction

Of the various functions of the larynx, which in essence is a valve-like constriction at the proximal end of the respiratory tract, the exploitation of the expiratory narrowing of the glottis for the purposes of phonation stands out as the most important in human behaviour since phonation is the principal mode of interpersonal communication. The bilaterally symmetrical tonic postural and phasic respiratory-linked motor-unit activity of the intrinsic laryngeal muscles during spontaneous breathing in lightly anaesthetised animals *(1,2)* and in unanaesthetised human subjects *(3,4)*, and the modulation of such activity during phonation *(2,5,6)*, are dependent not only on the respiratory-linked discharges of the laryngeal motoneurone pools located in the nucleus ambiguus in the medulla oblongata *(7,8)* but also on the afferent activity derived from a triad of intralaryngeal mechanoreceptor systems—namely, the articular, myotatic, and mucosal receptor systems *(1,2)*. But while a good deal is now known about the morphology and function of the laryngeal articular and myotatic receptor systems, there has as yet been no comparable systematic study of the subglottic mucosal receptor systems. A detailed morphological and functional investigation of the nerve endings in the subglottic mucosa of the larynx, designed to fill this gap, has been carried out in the Neurological Unit of the Royal College of Surgeons of England under the supervision of Dr B D Wyke and the results are reported here.

Morphological features of the innervation of the subglottic laryngeal mucosa

A detailed account of our morphological studies of the extrinsic and intrinsic innervation of the subglottic mucosa of the larynx has been provided in a paper published last year *(9)*, but the essential features of this innervation are summarised here.

EXTRINSIC INNERVATION OF THE SUBGLOTTIC MUCOSA

The extrinsic nerve supply of the subglottic region of the laryngeal mucosa was examined in a series of microdissection studies of the terminations of each of the laryngeal nerves in 16 cats. In essence, the results confirm the principal features of several previous anatomical and physiological investigations *(2, 10–13)* which show that the afferent nerve supply of most of the subglottic mucosa is provided through the recurrent laryngeal

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Arnott Demonstration given on 28th January 1980

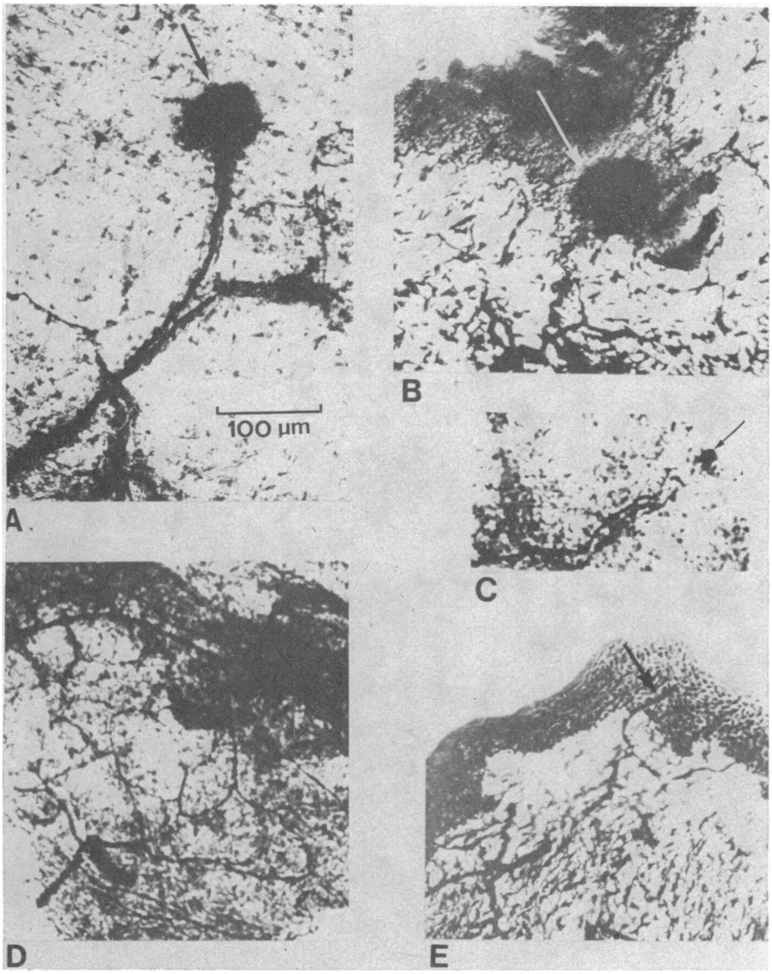

FIG. 1 *Nerve endings in laryngeal subglottic mucosa (magnification by scale). (A) Large globular corpuscle (arrowed) with its parent afferent axon embedded in the subepithelial mucosal tissue (gold chloride preparation). (B) Large globular corpuscle (arrowed) embedded in the deeper layers of the mucosal epithelium; its parent axon can be made out below the corpuscle (frozen silver preparation). (C) Small globular corpuscle (arrowed) with portion of its parent axon embedded in the subepithelial mucosal tissue (gold chloride preparation). (D) Plexiform nerve endings (mixed with blood vessels) in the submucosa (gold chloride preparation). (E) Epithelial surface of mucous membrane, showing (arrowed) intraepithelial nerve terminals (frozen silver preparation). (Reproduced by permission from* Folia Phoniatrica (Basel)) (9).

nerve. However, the external branch of the superior laryngeal nerve provides the innervation of the mucosa lining the cricothyroid membrane anteriorly (see also 2,9). It should be noted that the external laryngeal nerve has in fact now been shown to contain not only afferent fibres from the subglottic mucosa but also some of the afferents from the receptors embedded in the capsules of the cricothyroid joints; for this reason the traditional textbook statement that the external branch of the superior laryngeal nerve is a pure motor nerve is no longer tenable and should be abandoned.

INTRINSIC INNERVATION OF THE SUBGLOTTIC MUCOSA

The nature and distribution of the nerve endings in the subglottic mucosa were examined in 27 adult cat larynges freshly removed from anaesthetised animals. After laryngectomy the larynx was divided vertically in the midline in an anteroposterior plane and each half was pinned flat on a dissecting board with the mucosal surface uppermost. Using the operating microscope, the supraglottic larynx and the upper tracheal rings were separated from the intervening subglottic region, the mucosal lining of which was divided into seven zones by microdissection—one being the mucosa covering the inferior surfaces of the vocal folds—to facilitate a thorough histological exploration of the regional population density of the nerve endings in the mucous membrane. Each mucosal zone was then independently removed by microdissection from the underlying tissues, washed in physiological saline solution, and processed immediately with two neurohistological techniques—namely, the Wyke gold chloride procedure and the Fitzgerald frozen silver method.

With these procedures both corpuscular and non-corpuscular nerve endings were identified in the subglottic laryngeal mucosa.

Corpuscular nerve endings Three varieties of corpuscular nerve ending were observed, and these (in order of decreasing frequency of occurrence) were as follows:

a) Medium-sized (about 100 × 80 μm), thinly encapsulated globular corpuscles. These were innervated, either singly or in pairs, from a single parent myelinated axon (Fig. 1A) and were mainly distributed through the loose submucous tissue. A few, however, were embedded in the deeper layers of the epithelial lining of the mucosa (Fig. 1B).

b) Smaller (about 20 × 10 μm), thinly encapsulated globular corpuscles. These were innervated in clusters from a fine myelinated afferent nerve fibre and were randomly distributed throughout the loose submucous tissue (Fig. 1C). None were present in the epithelial lining of the mucous membrane.

c) Small conical corpuscles (measuring about 40 × 20 μm) with a multilaminated capsule. These were innervated by a small myelinated afferent axon, were much less numerous than the globular corpuscles, and were confined to the submucous tissue.

Non-corpuscular nerve endings These consisted of a plexus of fine unmyelinated nerve fibres (Fig. 1D) that ramified freely throughout the submucous tissue and from which free nerve endings (Fig. 1E) penetrated into the superficial epithelial layer of the subglottic mucosa, sometimes as far as its surface.

REGIONAL DISTRIBUTION OF NERVE ENDINGS

The population density of the corpuscular nerve endings varied in different regions of the subglottic mucosa. The mucosa covering the inferior surfaces of the vocal folds was most densely innervated (mainly with the medium-sized globular variety of corpuscle) and the density of the corpuscular innervation decreased therefrom in both rostrocaudal and anteroposterior directions, there being no corpuscular nerve endings in the thin mucosa overlying the cricoid cartilage posteriorly or in the inferior portions of the mucosa where it becomes continuous with that lining the trachea.

On the other hand the plexiform nerve endings appeared to be uniformly distributed throughout the entire submucosa of the subglottic larynx, including that covering the inferior surfaces of the vocal folds.

These neurohistological studies provide (for the first time) definitive evidence of the presence of a variety of receptor nerve endings in the mucous membrane lining the subglottic larynx that differ in population density and morphology (and hence in their functional characteristics) from region to region.

Reflexogenic functions of laryngeal subglottic mechanoreceptors

The fact that the subglottic mucosal mechanoreceptors exert significant reflexogenic effects on the laryngeal muscles has been demonstrated in multichannel electromyographic studies of the tonic postural and respiratory-linked phasic motor-unit activity of these muscles. The experiments were performed on 33 cats lightly anaesthetised with pentobarbitone and breathing an oxygen-enriched air mixture spontaneously either through the larynx (via a nasopharyngeal airway) or through a low-neck tracheostomy tube. Simultaneous bipolar electromyograms were recorded, using pairs of fine stainless steel needle electrodes, from three intrinsic laryngeal muscles (the thyroarytenoid, posterior cricoarytenoid, and cricothyroid muscles) and two extrinsic (sternothyroid and thyrohyoid) laryngeal muscles.

After an initial period of control recording, a 1% solution of lignocaine hydrochloride solution (Xylocaine) was carefully applied under direct vision to the surface of the subglottic mucosa through an upper tracheal incision. The incision was then closed with sutures and checked to be airtight, after which the electromyographic recordings were continued over the next 90 min. All exposed tissues were submerged in a pool of warm mineral oil to prevent their drying, and the rectal temperature of the animals was maintained between 37 and 38°C on a thermostatically controlled operating table. Con-

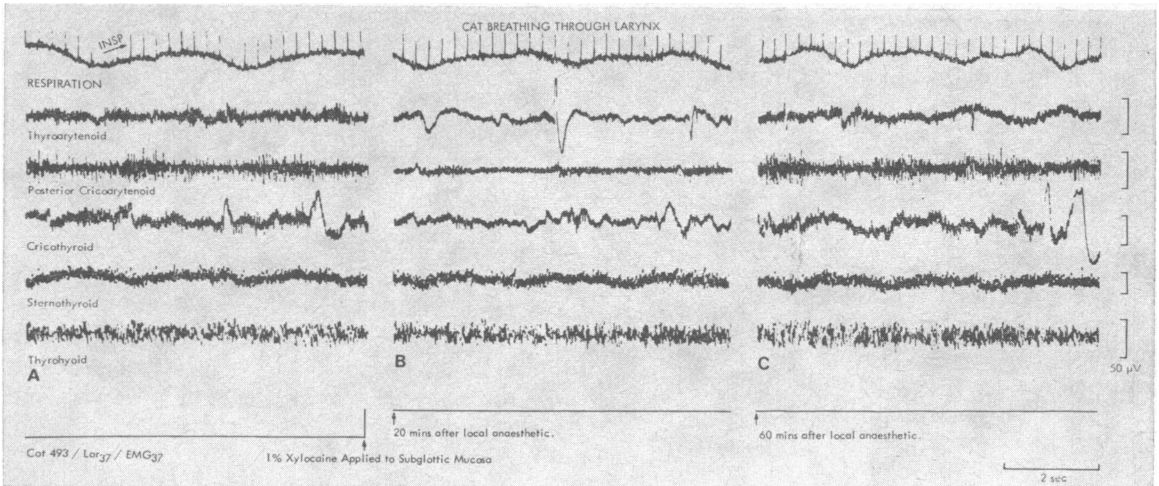

FIG. 2 *Simultaneous electromyograms of intrinsic and contralateral extrinsic laryngeal muscles in a lightly anaesthetised cat breathing spontaneously through the larynx (calibration on right). Respiratory cycle recording with superimposed electrocardiogram in upper tracings (Insp: inspiration upward, indicated by arrow). Description in text.*

current recordings were also made of the respiratory movements of the thorax and of the electrocardiogram, and the degree of anaesthesia was monitored and controlled from continuous recordings of the pulmonary ventilation rate.

Within 10–15 min of the topical application of anaesthetic to the subglottic mucosa there was a significant reduction in the tonic and phasic respiratory-linked activity of the intrinsic (but not the extrinsic) laryngeal musculature that persisted for 45–60 min (Fig. 2B) before control patterns of motor-unit activity (Fig. 2A) were re-established (Fig. 2C). Furthermore, when the respired airstream was diverted away from the larynx by allowing the animals to breathe through a tracheostomy tube there was a significant reduction in the motor-unit activity—and especially the phasic activity—of the intrinsic (but not the extrinsic) muscles of the larynx (compare Figures 3A and 3B).

It is important to emphasise that there was no significant change in the motor-unit activity of the extrinsic laryngeal muscles as a result of either topical anaesthesia of the subglottic mucosa or diversion of the respiratory airstream away from the larynx, which indicates that the reflexogenic influences of the subglottic mucosal mechanoreceptors are di-

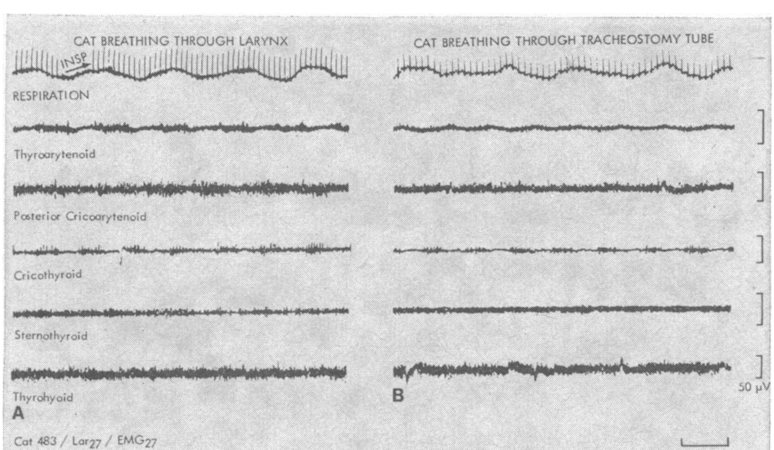

FIG. 3 *Simultaneous electromyograms of same laryngeal muscles as in Fig. 2 in a different lightly anaesthetised cat breathing spontaneously (A) through the larynx and (B) through a low tracheostomy tube (degree of general anaesthesia is the same in both circumstances). Upper tracings are respiratory cycle recordings with superimposed electrocardiogram (Insp: inspiration upwards, indicated by arrow). Description in text.*

rected exclusively at the motoneurone pools of the intrinsic laryngeal muscles. On the other hand it should also be noted (as the recordings in Figures 2 and 3 illustrate) that these reflexogenic effects are exerted both on the adductor and abductor components of the intrinsic musculature.

Discussion

Recent physiological studies *(14,15)* in which exposure of the laryngeal mucosa of cats to different concentrations of carbon dioxide, ammonia gas, riot control (CS) gas, and cigarette smoke was shown to provoke discharges in unmyelinated afferent fibres in the recurrent laryngeal nerve suggest that the subglottic laryngeal mucosa might contain chemoreceptor nerve endings sensitive to changes in the composition of the air passing over the laryngeal mucosa. In view of the superficial position of the intraepithelial free nerve endings described here and of the fact that these are innervated by unmyelinated afferent fibres it seems likely that the free nerve endings (as well as the submucous plexus) might function as the chemoreceptor system of the subglottic larynx. In this connection it should also be noted that stimulation of this laryngeal chemoreceptor system has been shown to modify reflexly the patterns of spontaneous breathing in experimental animals *(15–17)*.

It is further suggested that the corpuscular nerve endings that have been described in this communication represent a system of subglottic mucosal mechanoreceptors, the existence of which has been suspected for some time *(2,18)*. By analogy with the known behaviour of morphologically similar corpuscles in other tissues it might be expected that the globular corpuscles would operate as low-threshold, slowly adapting mechanoreceptors, whereas the conical corpuscles would function as low-threshold, rapidly adapting mechanoreceptors; and several neurophysiological studies *(2,11–13,19)*, involving various types of gentle mechanical stimulation of the subglottic mucosa, have shown that this tissue contains low-threshold mechanoreceptors with both slowly and rapidly adapting characteristics. The results of the present study indicate that afferent discharges from the low-threshold rapidly and slowly adapting mechanoreceptors that are present in the subglottic mucosa (and which are sensitive to changes in subglottic air pressure) exert significant reflex effects on the motor-unit activity of the intrinsic laryngeal musculature, even in the presence of the small fluctuations of subglottic air pressure (of the order of 2–3 cm H_2O (0.2–0.3 kPa)) that are associated with spontaneous breathing in lightly anaesthetised animals. This is evidenced by the fact that abolition of discharges from the mucosal receptors by topical anaesthesia or reduction thereof by diversion of the respiratory air stream out of the larynx produces significant changes in the tonic and phasic motor-unit activity of the intrinsic laryngeal muscles.

Because of their sensitivity to changes in subglottic air pressure it is further suggested (see also *2,6*) that the contribution of the laryngeal subglottic mucosal mechanoreceptors to the reflex control of intrinsic laryngeal muscle activity will assume increasing importance when the subglottic air pressure during expiration rises to levels above those that obtain during quiet breathing, as occurs in speaking and even more during singing *(2,5,6)*. This proposal is in fact supported by reports of the deleterious effects of topical anaesthesia of the subglottic laryngeal mucosa of human subjects on their speaking and singing voice *(20,21)*.

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