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Different glycoconjugates in the submucosal glands of the supraglottis and subglottis. Lectin histochemistry study in the hamster

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Abstract

A lectin histochemistry study was performed in the supraglottic and subglottic regions of 10 hamsters. The submucosal glands were observed by light microscopy. The supraglottic submucosal glands presented numerous mucous tubules but on the other hand, the subglottic submucosal glands had serous tubules which finished at the distal portion in serous acini. The results suggest that the distribution of fucosylated-mucin and serum-type glycloproteins between the supra- and subglottic submucosal glands suggest a different viscosity and function of the mucus.

Key words: Lectins; Laryngeal mucosa; Glycoproteins; *Mesocricetus auratus*

Introduction

Although there have been several studies on laryngeal submucosal glands secretion (Nielsen, 1988), differences between the glycoconjugates in these submucosal glands between the supra- and subglottic regions have not been reported (Pastor *et al.*, 1994). Lectins are proteins found in seeds and other structures of plants that have binding specifity for specific sugar moieties (Lalwani *et al.*, 1996).

The aim of this study was to investigate the differences between glycoconjugates and their distribution in the submucosal glands secretion in the larynx of the hamster. The mucus in the supraglottis may be used as a lubricant for vocal fold movement and protection whereas the quality of mucus in the subglottis could be used as a defence mechanism against bacteria and viruses, and, therefore, some qualitative differences could be expected between the two sites.

Materials and methods

Ten Syrian golden hamsters (*Mesocricetus auratus*) were used in this study. All were female and approximately 14 months old. The animals-use protocol was approved by the local committee of the laboratory animals-use and care. After the animals were sacrificed with a thiopental sodium, the larynx was removed and fixed and decalcified in a EDTA solution.

The specimens were routinely processed and embedded in paraffin and 5 μm sections cut. For lectin histochemistry, five sections from the supraglottis and five sections of the subglottis in each specimen were treated as previously reported (Pastor *et al.*, 1992). Endogenous peroxidase was blocked with 0.3 per cent H_2O_2 to destroy endogenous peroxidase activity. After washing in TBS (Tris buffered saline, pH: 7.4), the slides were incubated for two hours at room temperature in a moist chamber with horseradish peroxidase-conjugated lectins (Sigma, St Louis, MO) at the appropriate

TABLE I
LECTIN CHARACTERISTICS

Botanical name	Abbreviation	Concentration	Carbohydrate binding specificity
Arachis hypogaea	PNA	12 μg ml^{-1}	β-D-Gal(1-3)-D-GalNAc
Canavalia ensiformis	Con-A	20 μg ml^{-1}	α-D-Manosa
Dilochos biflorus	DBA	15 μg ml^{-1}	α-D-GalNAc
Glycine max	SBA	12 μg ml^{-1}	α-D-GalNAc
Lotus tetragonolobus	LTA	25 μg ml^{-1}	α-L-Fucosa
Triticum vulgaris	WGA	6 μg ml^{-1}	(β-D-GlcNAc)n, sialic acid
Ulex europaeus	UEA-1	25 μg ml^{-1}	α-L-Fucosa

Gal: Galactose; GalNAc: N-Acetylgalactosamine; GlcNAc: N-Acetylglucosamine.

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TABLE II
LECTIN HISTOCHEMISTRY CHARACTERISTICS OF THE SUBMUCOSAL GLANDS OF THE SUPRAGLOTTIS AND SUBGLOTTIS

	Supraglottis	Subglottis
HPA	+++	+++
N-WGA	++	+++
N-PNA	+++	++
WGA	++	++
DBA	−	+
SBA	−	+
PNA	−	+
UEA-l	+	−
ConA	−	+
LTA	−	−

(+++) strong, (++) moderate, (+) mild and (−) no reactivity.

dilution (Table I). After washing in TBS, peroxidase was developed in 0.05 per cent 3,3 diaminobenzidine tetrahydrochloride and 0.3 per cent H_2O_2 in TBS. When developed, the sections were dehydrated, cleared and mounted in DPX. Succinylation of WGA and PNA was made. The sections pretreated with neuraminidase to reduce the affinity to sialic-acid were N-PNA and N-WGA. Control for lectin staining included: substitution of lectin-peroxidase conjugates for TBS, and exposure of each lectin-peroxidase conjugate in the presence of a 0.2 M concentration of the corresponding inhibitory sugar (Sigma, St Louis, MO).

Results

There were submucosal glands in all the specimens studied in the supraglottic and subglottic regions. These submucosal glands were exclusively located at the base of the epiglottis and in the subglottis. The first presented numerous mucous tubules with some serous acini but the second glands had serous tubules which finished at the distal portion in serous acini. The serous tubules connected with a collector duct that also had some serous cells in its epithelium. Within the same animal all the specimens showed the same reactivity to the lectins and this was the same in the 10 animals.

Differences in reactivity to the lectins of the submucosal glands can be observed in Table II. Strong reactivity to HPA lectin was observed in the supraglottis and subglottis (Figure 1). Strong reactivity to N-WGA was observed in the subglottis while in the supraglottis strong reactivity to N-PNA was observed. Other differences were that, in the subglottis submucosal glands reactivity to DBA and SBA was observed but this was not seen in the supraglottis. UEA-l lectin reaction was observed in the supraglottis and ConA reaction was observed in the subglottis (Figure 2).

Discussion

Although the carbohydrate specifity of HPA-lectin is the same as DBA-lectin and very similar to that of SBA-lectin, their reaction with submucosal glands are quite different. There is no reaction to SBA-lectin and DBA-lectin in the supraglottis and it could be explained because these lectins may reflect the differences in the 'core' of the O-glycosidic saccharide chains of glycoproteins. However, UEA-l is a lectin specific for mucous secretions and it has also been considered a specific marker for the olfactory cells in different stages of development (Geri *et al.*, 1993) while the Con-A lectin reaction has only been observed in serous cells of the submucosal glands (Thaete *et al.*, 1981).

PNA have a high specifity for the disaccharide b-Gal(1-3)-D-GalNAc and WGA for GlcNAc(b-1,4-D-GlcNAc)1-2. The cellular glycoproteins are usually sialated and the sialic acid is the terminal residue on the glycoprotein side chain (Damjanov, 1987). However, subsequent addition of other terminal sugars masks the binding sites and makes the glycoproteins in the mucin droplets unreactive with these lectins. Removal of the terminal sialic acid with the neuraminidases (N-PNA and N-WGA) will expose the penultimate residues and make the glycoproteins reactive with PNA and WGA lectins. In the submucosal glands of the human larynx serous cells showed more affinity for N-PNA while mucous cells showed affinity for N-WGA (Pastor *et al.*, 1994).

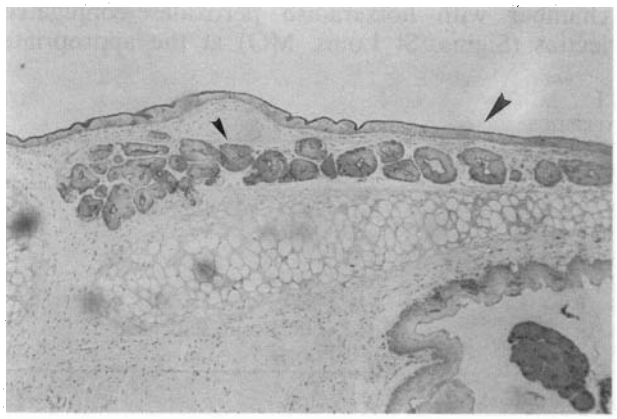

FIG. 1
Strong reactivity to HPA lectin in the SG (➤) and pericilliary layer (➤) of the base of the epiglottis (× 450).

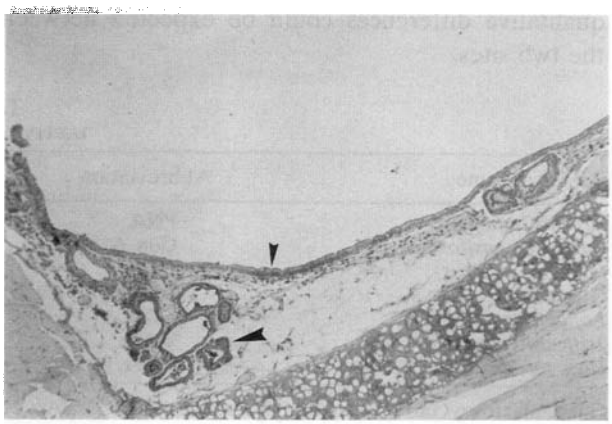

FIG. 2
Reactivity to Con-A lectin in the SG (➤), serous cells, of the subglottis (× 450).

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Glycoproteins are responsible for the specific properties of mucus-gel. The distribution of fucosylated-mucin and serum-type glycoproteins between supra- and subglottic submucosal glands suggest a different viscosity and function of the mucus. Lewis and Prentice (1980), have suggested that in the rat the mucus secretions of the submucosal glands in the epiglottis could aid mechanoreceptors and chemoreceptors in the expulsion of particulates in inspired air while in the subglottis they could be beneficial in defence against bacteria and viruses.

In conclusion, there are histochemical differences between submucosal gland secretions of the supraglottic and subglottic regions and this suggests different properties and functions of the mucus secretions within the hamster larynx.

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