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Postnatal morphological and lectin histochemical observation of the submucosal glands of rat nasopharynx

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ABSTRACT

The development of submucosal glands of rat nasopharynx was studied with respect to their morphological maturation and glycoprotein alterations during the postnatal period. This study examined the histological morphology with hematoxylin–eosin and the binding pattern of lectins, soybean agglutinin (SBA), *Dolichos biflorus agglutinin* (DBA), *Vicia villosa agglutinin* (VVA), *Ulex europaeus agglutinin*-I (UEA-I), peanut agglutinin (PNA), wheat germ agglutinin (WGA), and succinylated WGA (sucWGA) on frozen sections from newborn into adulthood. At birth, nasopharyngeal glands consisted of rudimentary secretory units which by postnatal day 3 (PN3) showed the characteristic features of salivary glands comprised of mixed mucous and serous cells. With maturation, serous cells increased in number and were arranged in clusters. Lectin reactivity at birth was detected at the acinar cell basal membranes for DBA, SBA, VVA, UEA-1 and PNA. At PN3, lectins labeled the apical cytoplasm and basolateral membranes of mucous cells and progressively with maturation, extended from the apical to basal portions of the cytoplasm with variable reactivity of VVA, PNA and sucWGA. Serous cells were labeled by UEA-1 starting from PN10 and also by PNA in adults. Ducts showed variable lectin reaction on the luminal membrane with strong reactivity of DBA and UEA-1 at PN21. Taken together, lectin histochemistry indicated the transitional occurrence of glycoproteins depending on the stage of maturation of the glands. Moreover, these results emphasize the difference in the morphology and lectin histochemistry between the nasopharyngeal and palatine glands.

1. Introduction

Minor salivary glands are spontaneous secretors that function to provide a protective mucous coat and act in maintaining a healthy balance between tissues and their environment (Amerongen et al., 1995; Tabak, 1995). There are approximately 1000 minor salivary glands distributed throughout the submucosa of the upper aerodigestive tract (Holsinger and Bui, 2007), and among them, the nasopharynx has a relatively low concentration of glands (Cao et al., 2012). Malignancies of minor salivary gland origin arising within the nasopharynx are uncommon and accounted for approximately 2% according to pooled data from two relatively large series that studied minor salivary gland malignancy (Ellis et al., 1988; Garden et al., 1994).

Nasopharyngeal glands are composed predominantly of serous cells, with a few mixed glands of separate mucous and serous acini, as well as mucous acini with serous demilunes (Ali, 1965; Tock and Tan, 1969). In humans, the first glandular primordia develop in the lateral wall of nasopharynx at the 12th gestational week. The glands then spread over a larger area in the nasopharynx – comprising the posterior wall and part of the roof of the nasopharynx, upper half of the pharynx, and also a portion of the soft palate and uvula – and encircle the Eustachian tube (Tos, 1970). To our knowledge, the development of nasopharyngeal submucosal glands in rats has been poorly documented.

Glycoproteins play an important role in many biological phenomena, such as, cell recognition, differentiation, and host-parasite interaction (Brockhausen et al., 1998; Wu, 2003). Comparative studies have shown that the abundance, distribution, and contents of secretory cells vary considerably among different species (Spicer and Schulte, 1992). Earlier conventional carbohydrate histochemical studies revealed that glycoproteins produced from nasopharyngeal submucosal glands of human (Tock and Tan, 1969), monkey and gibbon (Leela and Kanagasuntheram, 1973) are acidic, being made of sialomucins and sulfomucins, and a variable amount

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of neutral mucins. However, non-sulfated mucins are secreted predominantly in the nasopharyngeal glands of rodents (Spicer, 1960).

Lectins are proteins of non-immune origin which possess affinity for specific carbohydrate residues in glycoproteins and glycolipids. The use of lectins in histochemical studies on several tissues has provided valuable biological information about the cytological alteration of glycoconjugates during development and under different physiological and pathological conditions (Rademacher et al., 1988; Spicer and Schulte, 1992). Moreover, Lectin histochemistry has distinguished glycoconjugates considered to be related to an area of specialized physiological role and local activity (Spicer, 1993). Development of submucosal nasopharyngeal glands has been scarcely studied, with no existing work about their development and lectin histochemistry. Therefore, we conducted this study to investigate the morphological changes and glycoprotein alterations of nasopharyngeal glands during the postnatal period of rat development by means of lectin histochemistry.

2. Materials and methods

2.1. Animals and tissue preparation

All experiments performed for this investigation were reviewed and approved by Osaka University, Graduate School of Dentistry, Intramural Animal Use and Care Committee. Sprague-Dawley rats of both sexes at various ages and intervals were used. Following our previous study (Hakami et al., 2014), rats were divided into 4 postnatal developmental stages; suckling (PN0, 3, 7), transitional (PN10, 14), and weaning (PN21) stages and adulthood (PN56). A total of 26 rats was used; PN0 (4), PN3 (4), PN7 (3), PN10 (4), PN14 (3), PN21 (4) and PN56 (4). The animals were deeply anesthetized with chloral hydrate (600 mg/kg body weight, i.p.) and perfused transcardially with 0.02 M phosphate-buffered saline (PBS; pH 7.2) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The heads were fixed in 4% paraformaldehyde in 0.1 M PB (pH 7.4) for 3 days, and decalcified with 7.5% ethylene diamine tetraacetic acid (EDTA) for 1–4 weeks at 4 °C. After decalcification, the heads were cut into exact halves at the median plane. For frozen sections two heads for each group were transferred to PBS containing 20% sucrose. For paraffin sections the remaining heads were post-fixed in 4% paraformaldehyde in 0.1 M PB (pH 7.4) for at least overnight, and the specimens were dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin. In order to be more consistent we selected a specific area from the nasopharynx which is the lateral wall of the nasopharynx located above the posterior portion of the soft palate as indicated by the dotted area in Fig. 1.

2.2. Lectin histochemistry

We applied lectin histochemistry to frozen sections to avoid false negative error. Parasagittal frozen sections were prepared at a 14 μm thickness, and were mounted on MS-coated glass slides (Matsunami, Osaka, Japan), rinsed with PBS after they were dried, and processed for lectin histochemistry. For identification of specific carbohydrate residues, tissue sections were incubated for 30 min with 0.3% H_2O_2 in methanol to block endogenous peroxidase activity. The sections were then incubated with one of the 7 different biotinylated lectins (Table 1; Vector Laboratories, Burlingame, CA, USA) for 12–14 h and then washed three times in PBS, followed by processing with ABC (Vector Laboratories) for 60 min and washed again three times with PBS. Lectin binding sites were visualized by incubating the slides with 0.05 M Tris–HCl buffer (pH 7.5) containing 0.08% diaminobenzidine and 0.003%H_2O_2. The sections were slightly counterstained with Methyl blue, dehydrated and coverslipped with Permount (Fisher, NJ), and examined in a light microscope. Paraffin sections were cut at 7 μm, deparaffinized with xylene and rehydrated. Sections from each sample were stained with Hematoxylin and Eosin for general histological observation.

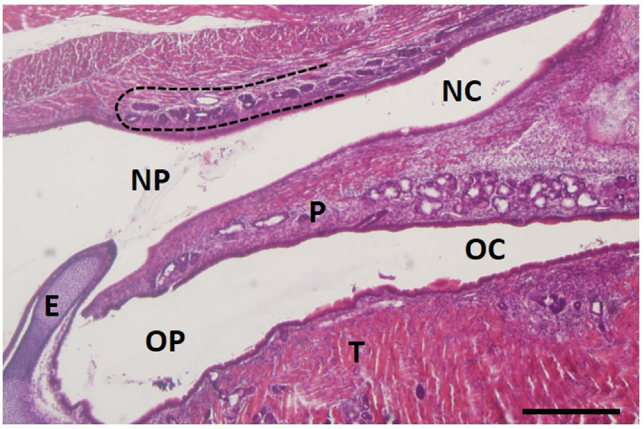

Fig. 1. Sagittal view of rat oral cavity and nasopharynx at birth. Note the distribution of secretory units in the submucosa of nasopharynx (dotted area). OC, oral cavity; NC, nasal cavity; P, palate; T, tongue; OP, oropharynx; NP, nasopharynx; E, epiglottis. Scale bar: 200 μm.

Table 1
Lectins and their binding specificities used in the present study.

Souce (common name)	abbreviation	Carbohydrate binding specificity
Glycine max (soybean)	SBA	Terminal α,βGalNAc > α,βGal
Dolicibos biflorus (horse gram)	DBA	Terminal FP > GalNac α1,3GalNac > GalNAc α1,3Gal
Vicia villosa (hairy vetch)	VVA	Terminal GalNAc α 1,3 Gal > GalNAc α1,6 Gal = GalNAc-Serine
Ulex europaeus-I (gorse seed)	UEA-I	α Fucose
Arachis hypogaea (peanut)	PNA	Terminal Gal β 1,3GalNAc
Triticum vulgare (wheat germ)	WGA	GlcNAc(β1,4GlcNAc)$_{1-2}$ >β GlcNAc > NeuAc
Succinylated WGA (wheat germ)	sucWGA	GlcNAc(β 1,4GlcNAc)$_{1-2}$

GalNAc = *N*-acetylgalactosamine, GlcNAc = *N*-acetylglucosamine, Gal = galactose. FP = Forssman pentasaccharide GalNAcα1,3GalNAcα1,3Galβ1,4Galβ1,4GlcNAc.

3. Results

3.1. Suckling stage (PN0-7)

In the newborn rat, abundant submucosal glands were distributed along the anterior, posterior and lateral wall of the nasopharynx, passing through the levator cushion in the region of the pharyngeal ostium (Fig. 1). At PN0, the secretory units were in a relatively rudimentary state. Lobulation was recognized and composed of immature acini and ducts that were separated by loose connective tissue (Fig. 2A). Ducts were observed with large and irregular lumens. These ducts in turn are continuous with branching terminal tubules. They are composed of simple cuboidal epithelium resting on a peripheral layer of flattened cells, probably myoepithelial cells. The duct cells are so small that their nuclei appear crowded together (asterisk in Fig. 2A, inset). The excretory ducts appeared to have a similar type of epithelium (asterisk in Fig. 3A, inset). At PN3, the secretory units of mixed mucous and serous acini demonstrated features characteristic of more mature acini. The mucous acini contained basophilic nuclei located basally

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