Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Distribution of nerve fibers during the development of palatine glands in rats

Zaki Hakami

Department of Preventive Dental Sciences, Division of Orthodontics, College of Dentistry, Jazan University, Jazan, Saudi Arabia

ARTICLE INFO

Keywords:
Palatine glands
Development
Innervation
Autonomic nervous system
PGP 9.5
GAP-43
CGRP
NPY

ABSTRACT

Background: Salivary gland maturation and function are modulated by the nervous system. Nevertheless, little is known about salivary gland innervation during development, particularly minor salivary glands. This study investigated the development of the innervation of the palatine glands of rat.

Materials and methods: Frozen sections of rat palatine glands at different stages were immunohistochemically labeled for detection of the general nerve markers protein gene product 9.5 (PGP 9.5) and growth associated protein 43 (GAP-43), and the autonomic nerve markers calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY).

Results: PGP 9.5 and GAP-43-immunoreactive fibers (IRF) were present in the mesenchyme and in association with developing acini, ducts and blood vessels. GAP-43-IRF were more abundant and diffuse than PGP 9.5-IRF at early stages, but showed similar distribution with growth, ramifying out from thick bundles in connective tissues until encircling the secretory units observed around postnatal day 21 (PN21). CGRP-IRF were detected in the mesenchyme at embryonic day 20 (E20) and PN0. CGRP-IRF became numerous around PN7 and PN10. They then decreased to the adult level at PN21, mainly located around ducts and infrequently blood vessels. NPY-IRF were sparsely detected in the mesenchyme at E20, then detected in close proximity to acini in addition to blood vessels at PN3. NPY-IRF increased till reaching the adult stage, and were mainly associated with blood vessels and around mucous cells and some serous demilunes.

Conclusion: The findings indicated a developmental modification of the sensory and autonomic innervation which may play a role in the functional maturation of the palatine salivary glands.

1. Introduction

Salivary gland function is a nerve mediated process that is controlled and influenced by parasympathetic and sympathetic autonomic nerves which work together harmoniously to evoke secretion (Proctor and Carpenter, 2007). It has been reported that parasympathectomy at birth causes marked atrophy of salivary glands (Kanno et al., 1987; Murakami et al., 1991). Moreover, sympathectomy at birth delays the development of acinar cells and granular duct cells causing reduction in salivary gland size (Henriksson et al., 1985; Srinivasan and Chang, 1977). Thus, in addition to regulating salivary gland secretion, the autonomic nervous system may play a role in organogenesis during development and glandular regeneration following diseases (Ferreira and Hoffman, 2013).

Local release of neurotransmitters or neuropeptides from autonomic nerve fibers is known to play a complementary role in the regulation of salivary secretion as well as blood flow and contents (Ekström, 1987; Ferreira and Hoffman, 2013). Several studies have shown changes of neuropeptide-containing nerve fibers during the development of major salivary glands (Kusakabe et al., 1996; Salo et al., 1993a, 1995). It also has been demonstrated that the density of the different neuropeptide-containing nerves is changed in the pathogenesis of the minor labial salivary glands of patients with Sjögren's syndrome (Batbayar et al., 2004; Fehér et al., 1999; Konttinen et al., 1992). Apart from that, less attention has been paid to the innervation of minor salivary glands during development.

Although minor salivary glands produce less volume than major salivary glands, the subjective sensation of dry mouth has been suggested to be related to the decreased minor glands salivation because of the abundant mucins secreted semi-continuously by the these glands, which are widely distributed throughout the oral mucosa (Eliasson et al., 2009; Eliasson and Carlén, 2010; Won et al., 2001). Salivary gland tumors account for 3% to 10% of all head and neck neoplasms and 10–25% of them originate in minor salivary glands (Eveson and Cawson, 1985). The palate is considered the most common location of both benign and malignant minor salivary gland tumors (Mejía-Velázquez et al., 2012; Pires et al., 2007). Nevertheless, compared with labial glands, palatine glands have received relatively little attention in the past, perhaps owing to their difficult accessibility (Izutsu et al., 1987).

E-mail address: zhakami@jazanu.edu.sa.

https://doi.org/10.1016/j.acthis.2017.12.007
Received 18 September 2017; Received in revised form 19 December 2017; Accepted 20 December 2017

Palatine glands are located beneath the soft palate, and mixed glands of predominantly mucus acini and few serous demilunes exist at the deep termination of the secretory units that irregularly grow by pouching (Leeson and Leeson, 1968; Nakamura et al., 2001; Srivastava and Vyas, 1979). In humans, these structures develop from a solid epithelial cord arising from the epithelial lining of the soft palate during week 11 of fetal life, and thereafter undergo lumenization, branching, and acinar differentiation (Nielsen and Westergaard, 1971). In rats, histological and morphological maturation of the developing palatine glands have been described in several studies (Hakami et al., 2014; Nakamura et al., 2001; Shinzato et al., 2004; Srivastava and Vyas, 1979). Recently, the development of palatine glands has been examined by lectin histochemistry, which has shown a transitional alteration of secretory glycoproteins depending on the stage of maturation of the glands as well as heterogeneous distribution of glycoconjugates between the posterior and anterior regions of the palatine glands (Hakami et al., 2014; Hakami and Wakisaka, 2016). In contrast, there is no work that addresses the innervation of the developing palatine glands in any mammalian species. Therefore, this study aimed to examine the entire innervation of developing rat palatine glands using protein gene product 9.5 (PGP 9.5) and growth-associated protein-43 (GAP-43), and also to evaluate the developmental behavior of two autonomic neuropeptides; calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY).

2. Material and methods

2.1. Animals

All experiments performed for this investigation were reviewed and approved by Osaka University, Graduate School of Dentistry, Intramural Animal Use and Care Committee prior to the onset of the study. Sprague-Dawley rats of various ages were purchased from Nihon Dohbutsu (Osaka, Japan). The day on which a vaginal plague was identified was designated as “embryonic day (E) 0” and the day of birth was designated as “postnatal day (PN) 0”. A total of 30 rats was used; E20 (3), PN0 (4), PN3 (4), PN7 (4), PN10 (4), PN14 (3), PN21 (4) and PN42 (4).

2.2. Tissue preparation

The pregnant rat was sacrificed by an overdose injection of chloral hydrate (800 mg/kg body weight, i.p.), and the fetuses extracted by Caesarian surgery. The whole heads were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4) for 3 d. For postnatal experiments, 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), and then by 4% paraformaldehyde in 0.1 M PB. The head was further fixed in the same fixative for 3 d and then decalcified with 7.5% ethylene diamine tetraacetic acid (EDTA) for 1–4 weeks at 4 °C. After decalcification, the head was cut into exact halves along the median plane and soaked in PBS containing 20% sucrose. Parasagittal frozen sections were prepared at a thickness of 14 μm and submitted for immunohistochemistry. For general histological observations, specimens were embedded in paraffin, cut to 7 μm thickness, and stained with hematoxylin and eosin (HE).

2.3. Immunohistochemistry

The procedures were conducted as described previously (Honma et al., 2017; Miki et al., 2015). Sections were rinsed in 0.02 M PBS several times and treated with absolute methanol containing 0.03% H_2O_2 for 30 min at room temperature to block endogenous peroxidase activity. After preincubation with PBS containing 3% normal swine serum (Vector Laboratories, Burlingame, CA, USA) and 1% bovine serum albumin (Sigma, St. Louis, MO, USA) for 30 min to reduce nonspecific binding, the sections were treated with one of the primary antibodies overnight at room temperature. Primary antibodies were as follows: rabbit polyclonal anti-PGP 9.5 (1:5000; UltraClone, Isle of Wight, England, #RA95101), rabbit polyclonal anti-GAP-43 (1:3000; Chemicon, Temecula, CA, USA, #AB5220), rabbit polyclonal anti-CGRP (1:3000; Sigma, #PC205L) and rabbit polyclonal anti-NPY (1:3000; Chemicon, #AB1915). Following several rinses in PBS, the sections were incubated with biotinylated swine anti-rabbit IgG (1:500; DAKO, Copenhagen, Denmark, E0353), and subsequently with ABC complex (Vector Laboratories). The antigen-antibody complexes were visualized by incubation in 0.05 M Tris-HCl buffer (pH 7.5) containing 0.08% diaminobenzidine and 0.003% H_2O_2. The reactions were intensified by 0.04% nickel ammonium sulfate. The immunostained sections were lightly counterstained with methyl blue, dehydrated, coverslipped with Permount (Fisher Scientific Inc., Fairlawn, NJ, USA) and examined under a light microscope.

Immunohistochemical controls were performed with omission of the primary antibody, secondary antibody, or ABC complex. They did not display any positive immunoreactivities.

3. Results

3.1. PGP 9.5 immunoreactivity

In E20, palatine glands contained of parenchyma of immature

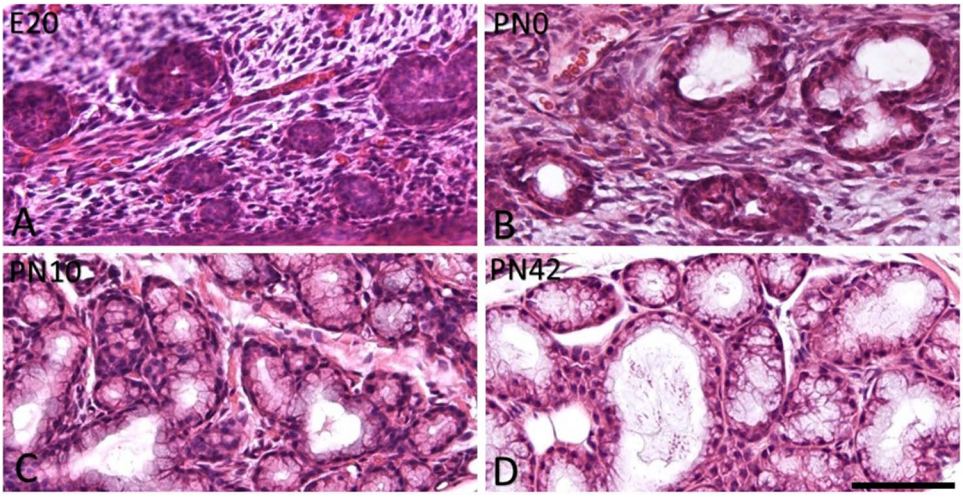

Fig. 1. Morphology of the developing rat palatine glands (HE). (A) Immature acini and ducts are formed at E20. (B) The secretory units are still sparsely distributed and separated by loose connective tissue at PN0. (C) Mucous cells gathered and are arranged in lobules at PN10. (D) In adults, glands are compact as a result of the increase in size and number of the acinar cells along with a reduction in the amount of stromal connective tissues. Scale bar in E: 50 μm for all parts of the figure.