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Structure of the secretory cells of male and female adult guinea pigs Harderian gland

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ABSTRACT

The main objective of this study was to investigate the structure of the Harderian gland (HG) in male and female guinea pigs. A total number of sixteen animals of 4 months age were divided according to sex into two groups; eight animals each. Unfixed glands were weighed and their length and width were measured. Specimens from fixed glands were processed and examined using light, transmission electron microscopy and immunohistochemistry for the detection of the presence of chromogranin A (CgA).

The gland consisted of a well-developed duct system which included both intra and extra parenchymal ducts and secretory end pieces lined by many types of cells of variable morphological features and modes of secretion. However, the holocrine mode of secretion was rare as mitotic figures were occasionally present. The interstitial cells included fibroblasts and immune cells (mast cells, lymphocyte, plasma cells and macrophages). The secretion produced by the gland included lipid, protein, neutral mucin and CgA which may be a newly identified constituent of biologically potent proteins stored in the cells of the guinea pig HG. Neutral mucin and CgA may function in photoprotection. The gland revealed sexual dimorphism in mast cells and blood capillaries number and chromogranin secretory activity.

1. Introduction

The Harderian gland (HG) fills the posterior part of the orbit and is closely attached to the posterior eyeball (Sakai, 1981). It is an exocrine gland located behind the ocular bulb in many mammals (Satoh et al., 1996). In rodents this gland is large and contains a considerable amount of lipid and porphyrins secreted via a duct that opens directly into the nictitating membrane (Watanabe, 1980).

The known functions of this gland include: lubrication of the eye, secretion of pheromones, secretion of growth factors, osmoregulation, photoprotection and thermoregulation (Chieff et al., 1993). The correlation between the possession of the Harderian gland and the presence of a nictitating membrane lead to the suggestion of the particular function of providing lubrication between the nictitating membrane and the cornea, however, this is not absolute as some animals like the guinea pig have well developed HG while they have no nictitating membrane (Smelser, 1943).

Although the HGs of different rodent species share many common features, none are identical. Besides, a minority scientific opinion contradicts the traditional classification of the guinea pig into Rodentia, proposing that caviomorphs, such as guinea pigs, chinchillas, and degus, are not rodents and should be reclassified as a separate order of mammals (Graur et al., 1991; D'Erchia et al., 1996). Guinea pigs are social, living in the wild in small groups. They are known to be sensitive to temperature changes and they must be kept out of extreme heat or cold. Temperatures above 26 °C can cause heat stroke and below 15 °C can cause them to become chilled. Therefore, the guinea pigs HGs are of particular interest; although they have no nictitating membrane they possess well developed HGs which do not secrete porphyrin (Seyama et al., 1984). It has been reported that the gland functions may vary with species (Payne, 1994 in tetrapods; Djeridane, 1996 in rodents; Sakai, 1992 in mammals). However, very little is reported on the morphology of guinea pig HG. It is stated that each species description is different and the morphology of the gland in any unstudied species could not be inferred safely from existing reports (Payne, 1994). The first description of adult guinea pig HG was by Paule (1957); the subsequent papers were about lipid synthesis (Satoh et al., 1993). A brief description for the gross and light microscopic structure of the HG in the guinea pig, along with other glands associated with the eye, was reported by Gasser et al. (2011). Although the postnatal development of the guinea pig HG has been studied (Elgayar et al., 2015), no detailed reports are available on the structure of HG in adult guinea pig. Therefore, the lack of an in depth study prompted us to carry out this work which aimed at providing a detailed morphological study of secretory cells in

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male/female guinea pig HG using light, electron microscopy, and morphometric techniques. Besides, we investigated the possible expression of chromogranin A (CgA) using immunohistochemistry. Chromogranins are present not only in secretory vesicles of nervous and endocrine cells but also in immune cells (Helle, 2004) and exocrine cells of salivary glands (Saruta et al., 2005) and reported to be involved in the innate immunity response to bacteria, fungi, and yeasts by its highly cationic nature (Briolat et al., 2005). Understanding the glandular structure would help in further understanding its function.

2. Material and methods

A total number of sixteen adult pigmented guinea pig that were 4 months age, 450–600 g body weight were used and divided, according to sex, into two groups (eight animals each). The experiment was approved by the institutional ethics committee of Assiut University. Three animals from both sexes were anesthetized with ether, then the eye was excised and the gland was dissected out, weighed and measured in the vertical and horizontal planes. After anesthetization of the rest of the animals, they were perfused intracardialy with the appropriate fixative (5% glutaraldehyde for electron microscopy (EM) and 10% formalin for light microscopy (LM)).

2.1. LM

The specimens excised gland was immersed into 10% formalin for two days fixation, then processed for preparation of paraffin blocks. Paraffin sections (5–7 μm) were cut into a transverse plane, mounted into glass slides and every tenth section was stained with hematoxylin & eosin stain (Drury and Wallington, 1980). In addition, selected sections were processed for histochemical demonstration of polysaccharides using Alcian blue (AB) at pH 2.5 for acid mucosubstances and periodic acid Schiff (PAS) method for neutral mucosubstances and Masson's trichrome stain for green staining of mucin and collagen fibers, silver stain for reticular fibers and Verhoeff's stain for elastic fibers. For lipid detection, formal-calcium-fixed frozen sections (5–10 m) were stained with Sudan Black B. Processing and staining techniques were done according to Drury and Wallington (1980).

2.2A. EM

The gland was cut into about 20 specimens of 2 mm^2 in size, immersed in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 24 h and post fixed in 1% osmium tetroxide in phosphate buffer for 2 h.

2.2B. Transmission electron microscope (TEM)

Small specimens were rinsed in the same buffer, dehydrated with alcohol, cleared with propylene oxide and embedded in epon 812. Semi-thin sections (0.5–1 μm) were cut and stained with toluidine blue (TB) (Gupta, 1983) for examination on a light microscope. Ultrathin sections (500-800A) were cut from selected areas of the blocks and contrasted with uranyl acetate and lead citrate (Reynolds, 1963). These sections were observed with the transmission electron microscope (TEM)("Jeol" E.M.-100 CX11; Japanese electron optic laboratory, Tokyo, Japan).

2.3. Scanning electron microscopy (SEM)

Large specimens were dehydrated in a graded series of alcohols and liquid carbon dioxide was used to dry the specimens which were mounted on aluminum stubs, fixed in place with colloidal silver and sputter coated with gold (Naguro and Breipohl, 1982). A jeol (J.S.M-5400 LV; Japanese Electron Optic Laboratory, Japan) was used to view the specimens. Photographs were taken at 15 kV and photographed at 80 kV.

2.4. Immunohistochemistry

Expression of chromogranin A (CgA) was detected in formalin-fixed paraffin-embedded sections (5 μm). Sections were deparaffinized, rehydrated, digested with protease XXV (enzyme-induced epitope retrieval) at 1 mg/ml PBS solution for 10 min at 37 °C and processed according to the manufacture instructions using the universal kit (ultravision detection system, antipolyvalent, HRP/DAB, Thermo Fischer Scientific, Fremont, CA, USA). Immunohistochemical staining was demonstrated using labeled streptavidin-biotin immunoperoxide technique. Mouse monoclonal antibody Ab-1 Clone LK2H10 Lab Vision Corp, Neo Markers Inc/Lab Vision, Fremont, CA, USA) was used at 1:800 dilution for 20 min at room temperature. Sections were then incubated with biotinylated goat anti-polyvalent secondary antibody for 10 min at room temperature. The reaction was then visualized using di-aminobenzidine (DAB) for 10 min. Sections were counterstained using Mayer's hematoxylin, dehydrated and cover slipped using DPX. Slides prepared from human small intestine stained with anti Chromogranin-A monoclonal antibody were used as positive control. In negative control slides, the primary antibody was replaced by phosphate buffer solution.

2.5. Morphometry

Using computerized assisted image analysis, the number of mast cells and blood capillaries per field was counted in semi-thin sections stained by toluidine blue in male and female, viewed using ×100 oil immersion lens. Blood capillaries were defined as those structures lined by endothelial cells which might contain erythrocytes. Thirty fields were counted using the modified touch method (Vohra et al., 2002). The morphometric data of each group were statistically analyzed. The Student's *t*-test was employed to compare the studied animal groups. $P < 0.05$ was considered significant.

3. Results

3.1. Gross appearance of the HG

The HG of both male and female guinea pigs lies horizontally at the antero-ventral surface of the eye (Fig. 1A), horse shoe in shape with two limbs (anterior and posterior) and two ends (medial and lateral). The anterior limb extends medially ventral to the larimal gland (Fig. 1A). It is surrounded by a thin capsule and has a convex surface and a concave hilum from which an extra-parenchymal duct exits and extends from the side of the posterior limb to the nasal side of the conjunctiva (Fig. 1B, C). It has two poles which extend medial and lateral to the optic nerve (Fig. 1D). Its mass is 0.34 g in females (0.05% of the body weight), 1.7 cm in length and 0.4 cm in width and 0.39 g in males (0.05% of body weight), 2 cm in length and 0.5 cm in width.

3.2. Light and TEM

3.2.1. Duct system

The gland is divided by connective tissue septa into lobes and lobules containing secretory end pieces and a well-developed intra parenchymal duct system. The extra parenchymal ducts are lined by pseudostratified columnar epithelium with goblet cells which are PAS & AB positive (Fig. 1E, F). The intra parenchymal ducts include inter-calated or intra-lobular ducts which arise from the