



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Ultrastructure and histochemistry of the subepithelial glands of the nasal septal island in dromedaries with special reference to the possible functions

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ARTICLE INFO

Article history:

Received 12 April 2021

Revised 18 May 2021

Accepted 20 May 2021

Available online 24 May 2021

Keywords:

Camels

Dromedaries

Subepithelial glands

Nasal septal island

APUD cells

ABSTRACT

The nasal septal island (NSI) is a sensory patch of neuroepithelium located within the soft tissue of the nasal septum in dromedaries. The island has unique anatomical features, including the specialized subepithelial glands. The aim of the present study was to describe the microscopic features and ultrastructure of these subepithelial glands and to speculate the possible functions. A total of 10 camel heads were used for the study. Unlike the serous and mucous airway glands, the NSI glands' ultrastructural features were typical for cells of the (Amine Precursor Uptake and Decarboxylation, APUD) system. These features were included, membrane bound secretory vesicles of varying electron density, smooth endoplasmic reticulum in the form of vesicles; electron dense mitochondria, abundant rough endoplasmic reticulum and free ribosomes. Alcian-PAS identifiable mucus granules were not observed, except for few clusters of cells, located at the luminal surface. The probable functions were discussed on basis of cellular morphology and context. In a conclusion, the NSI subepithelial glands in dromedaries had unique anatomical structures, and as many other APUD cells, they had the machinery required for synthesis of a variable number of biologically active peptides, amines and chemical mediators.

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1. Introduction

The nasal septal island (NSI) in dromedaries is a unique anatomical structure, formed of a patch of sensory epithelium, located in the rostro-dorsal part of the nasal septum and innervated by the ethmoidal branch of the trigeminal nerve (Abo-Ahmed et al., 2021). Interestingly, this sensory island was associated with three sets of glands, including submucosal, subepithelial, and intraepithelial glands as described by the previous authors. Microscopically, the specialized glands located along the nasal cavity, such as the olfactory gland of Bowman's (Getchell and Getchell, 1992), and the glands of the nasal mucosa (Widdicombe and Wine, 2015) were entirely formed of mucous and/or serous glands.

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Peer review under responsibility of King Saud University.



However, during the preparation of our previous study, we noticed that the NSI subepithelial glands had a remarkably distinct anatomical structure. Notably, the NSI glandular cells were resemble the cells of the Amine Precursor Uptake and Decarboxylation system, in short, APUD system (Langley, 1994). This motivated the authors to establish the present study, to focus more closely on the anatomical features of these glands and to verify their identity.

The concept of APUD system was initially described by Pearse (1968, 1969) and recently coined by Fujita (1983) as paraneuronal system (Langley, 1994; Boyd, 2001). Paraneurons are receptor-secretory cells storing substances such as bioactive peptides, amines and a number of chemical-mediators that play a key role in homeostasis, metabolism and immune tolerance (Gunawardene et al., 2011). Unlike the ordinary secretory cells, the APUD cells are the largest endocrine system in vertebrate living body (Langley, 1994; Gunawardene et al., 2011). However, it still remains little understood.

The APUD cells are characterized by a special type of secretion, which is compatible with the diversity and complexity of the bioactive materials that these cells synthesize, store and secrete. This type of secretion is known as piecemeal degranulation, which is a complex model of exocytosis characterized by slow release of

vesicular materials without vesicles opening to the cell exterior (Crivellato et al., 2005). Most characteristically, cytoplasmic vesicles undergoing piecemeal degranulation exhibit various degree of content loss (Crivellato et al., 2003), and therefore appear heterogeneous, i.e., of various degree of density at electron microscopic level.

All the above-mentioned information, raises the question, why such type of cells would be important in the nasal mucosa, namely in the NSI subepithelial glands? This could be addressed by the topographical context, as they are located within the chemosensory field of the ethmoidal nerve, suggestive for pain perception (Abo-Ahmed et al., 2021). Similar to other parts of mucosa, the nasal mucosa requires coordinating immune response with detection of noxious stimuli. This may help the animal to assess and avoid harmful noxious and chemical irritants. In such location, the subepithelial glands may help detection of chemical noxious cues. In addition, it may also help regulation of pain perception through the vast cascade of mediators and compounds they are able to release.

To the best of the author's knowledge, the available literature did not declare any information regarding the presence of a specialized gland in such location in any mammalian species. Therefore, the aim of the study was to describe in detail the microscopic, histochemical and ultrastructural features of the glandular components, using light and transmission electron microscopy, and to speculate the possible functions.

2. Materials and methods

2.1. Samples

The study was approved by the committee of scientific research ethics, Faculty of Veterinary Medicine, Benha University, Egypt. The current study was carried out on 10 paired organs from ten

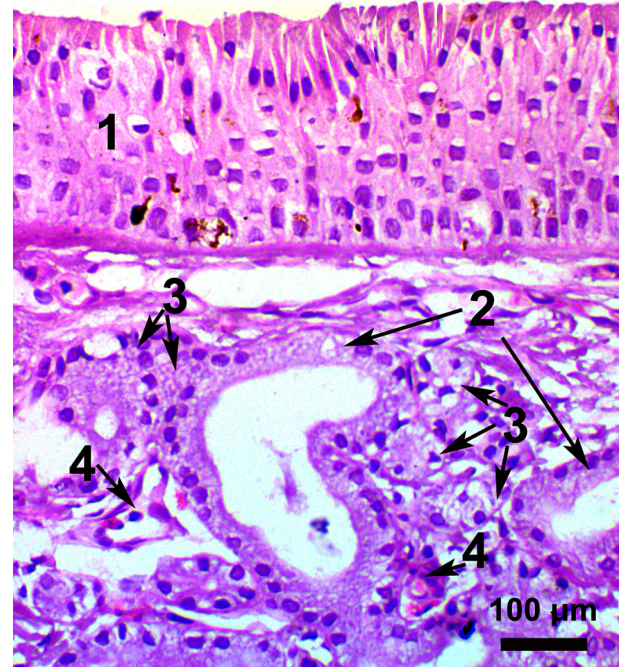


Fig. 2. Light micrograph showing cross section in the nasal septal island in dromedaries; stain H&E, X 100: neuroepithelium (1), the alveolar portion (2) of the subepithelial glands, which surrounded by the narrow lumen acini (3), and blood capillaries (4).

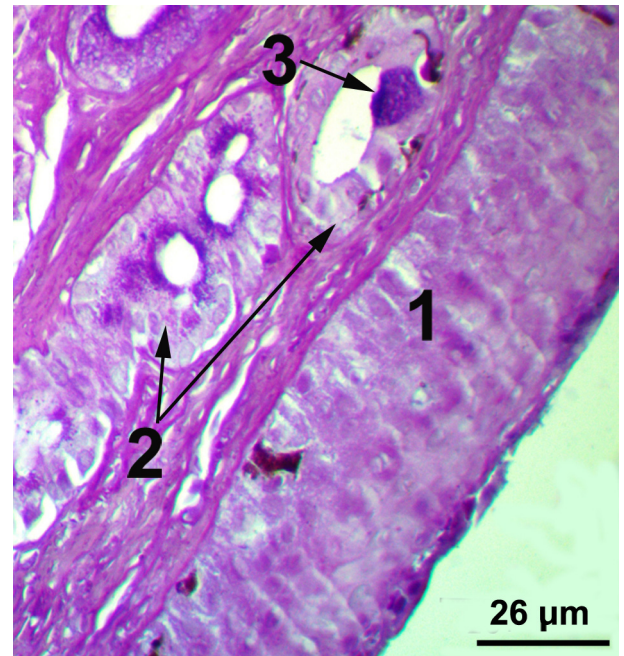


Fig. 3. Light micrograph showing the subepithelial glands stained with Alcian & PAS, X 400: neuroepithelium (1), subepithelial glandular cells exhibiting slight reaction only with PAS (2), luminal clusters showing positive reaction with Alcian & PAS (3).

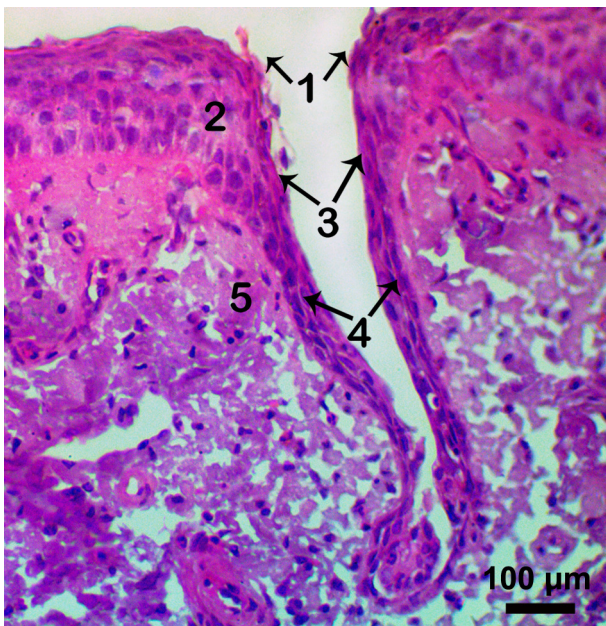


Fig. 1. Light micrograph showing sagittal section in the nasal septal island in dromedaries, stain H&E, X 100: A subepithelial gland opens onto the surface epithelium through a pore (1), which located within the neuroepithelium (2), and opens into an intraepithelial duct or canal (3), leading to an alveolar portion (4) deeply into the lamina propria (5).

3–6 years old apparently healthy mature male dromedaries (*Camelus dromedarius*). Tissue samples were immediately collected after slaughter from Qalioube and Toukh abattoirs, Egypt. Any abnormalities of the nasal septum were excluded.

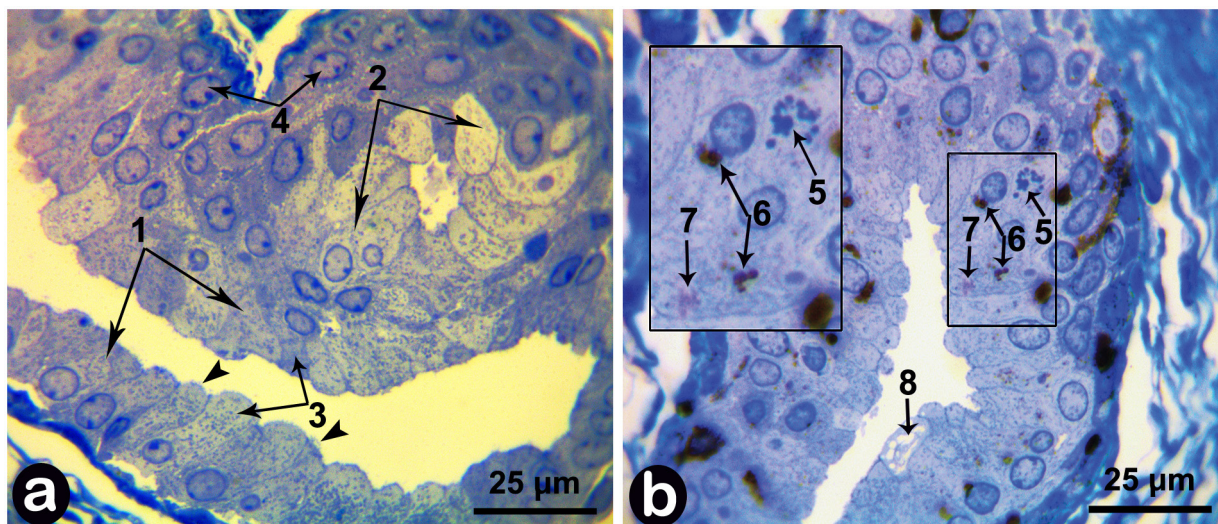


Fig. 4. Semithin section in the subepithelial gland (a, b), Toluidine blue, X 1000 showing: Alveolar portion (1), acinar portion (2), luminal columnar microvillar cells (3), non-luminal or closed basal cells (4), bluish granules (acidic protein) (5), yellowish-copper granules characteristic for APUD cells (6), purple colored granules, an indicator for the catecholamine (7), luminal clusters of vacuolated cells (8), apical microvilli (black arrow head).

2.2. Light microscopy (LM)

Histological specimens were immediately collected after slaughtering the animals, transected to obtain sagittal, transverse and oblique sections. The tissue specimens were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol (70–100%), cleared in xylene and embedded in paraffin. Three µm sections were mounted and stained with hematoxylin and eosin (H&E), and combined Alcian blue pH 2.5, Periodic acid Schiff (PAS) techniques for neutral and acid mucins, respectively (Bancroft and Stevens, 1996). Stained histological sections were examined and photographed using light microscope (Optika B-193, Italy) with a digital camera, X100–1000.

2.3. Transmission electron microscopy (TEM)

Small segments of fresh specimens were fixed in 4% buffered glutaraldehyde, pH 7.2, for 3 h at 4 °C. The tissue segments were post-fixed in 1% osmium tetroxide, dehydrated in graded series of acetone at 4 °C, embedded in resin and cut into sections about 0.009 µm in thickness. Staining of semithin sections was conducted with 1% toluidine blue and 1% sodium borate (borax) in 100 ml distilled water. Ultrathin sections were contrasted with uranyl acetate for 5 min, followed by lead citrate for 2 min and examined using electron microscopy JEOL-JSM-1400 PLUS, (Tokyo, Japan) at X800–X4000 in the electron microscopy unit, Alexandria University, Egypt.

3. Results

3.1. Microscopic structure

In the sagittal thick sections, the subepithelial glands appeared like pits or pouches within the neuroepithelium of the nasal septal island (Fig. 1). Each pit opened onto the surface through a pore, leading to an intraepithelial duct or canal, which continued distally within the lamina propria, forming an alveolar portion (Fig. 1). The single alveolus was consisted of a bulbous acinus with a dilated lumen that lies in contact with the basement membrane of the surface epithelium (Fig. 1). In the lamina propria, each alveolus was surrounded by numerous narrow lumened acini (Fig. 2). Notably,

some blood capillaries were in close proximity to the acinar basement membrane (Fig. 2). Both the alveoli and acini were lined with a series of columnar to pyramidal glandular cells, pale stained with H&E. They also reacted slightly, except for isolated cells only with PAS (Fig. 3). Some luminal clusters of secretory cells were found within the alveolar epithelium, and reacted positively with Alcian blue and PAS (Fig. 3).

In the semithin sections, the alveoli and acini of the subepithelial glands were consisted of two types of cells, the luminal columnar microvillar cells, and the non-luminal or closed basal cells (Fig. 4a, b) in addition to some luminal clusters of vacuolated cells that were observed only in the alveolar portion (Fig. 4b). Numerous granules (vesicles) were observed in the columnar and basal cells, including, the characteristic yellowish–copper APUD granules, reddish- purple (catecholamine) granules, and bluish (acidic protein) granules (Fig. 4b). At LM level, no intraglandular ducts were observed between the acinar and the alveolar portions.

3.2. Ultrastructure

The luminal cells of the subepithelial glands were characterized by the presence of heterogenous secretory vesicles, i.e. of variable size and electron density (Fig. 5a). Mostly, these vesicles exhibited piecemeal degranulation, showing loss of their contents without being shuttled to the cell membrane or fused with it (Fig. 5a). Large electron lucent vesicles, small neuron-like dense core vesicles, and large vesicles of moderate electron density were observed (Fig. 5a). The latter had a characteristic intra-vesicular electron-dense domains and semilunar chambers (Fig. 5a). While, the granular content of the small dense core vesicles was loosely packed, and some of these electron-dense granules were seen within the cytoplasm of the upper cellular region, out of their vesicles (Fig. 5a).

The luminal glandular cells had a complex network of rough endoplasmic reticulum, and well-developed intracellular canaliculi (Fig. 5a). The apical domain of these cells was short microvilli that extended towards the luminal surface (Fig. 5a, b). Some of these microvillar cells were pyramidal in shape with vacuolated cytoplasm, while the columnar microvillar cells were the dominant cells (Fig. 5c). Small, round basal cells were residing on the basement membrane and never reach the luminal surface (Fig. 5c).

These microvillar cells had oblong to round euchromatic nuclei. The prenuclear region of the columnar microvillar cells was rich in

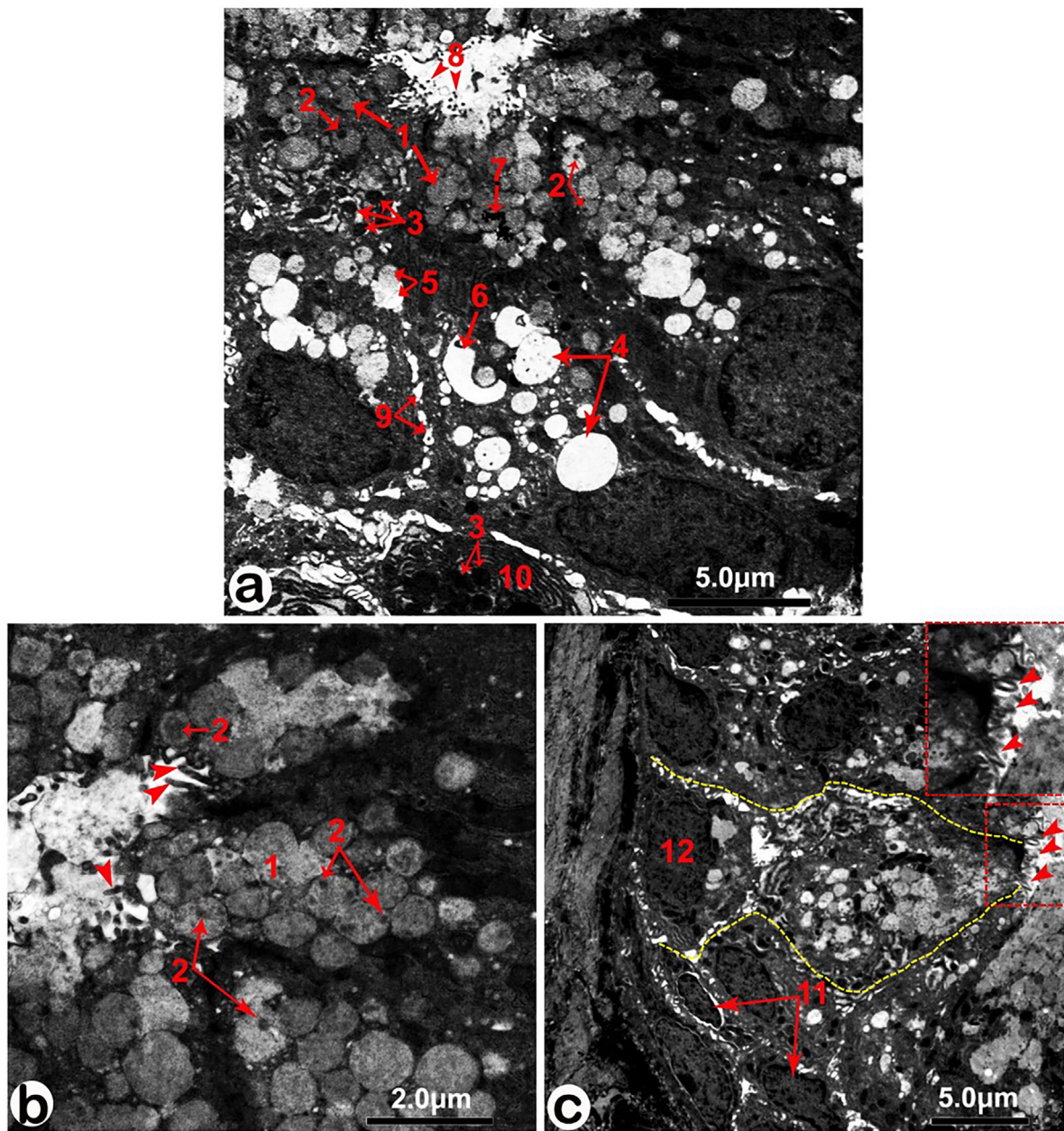


Fig. 5. Ultrathin section of the acinar portion of the subepithelial glands, X 1500 (a), X 4000 (b), X 1200 (c), showing features of piecemeal degranulation: secretory vesicles of moderate electron density (1) exhibiting characteristic electron-dense domains within the vesicles chambers (2), small neuron-like dense cored vesicles (3), large electron lucent vesicles (4), vesicles showing loss of its content (5), semilunar vesicular chamber (6), electron dense granules out of their vesicles (7), secretory granules within the lumen (8), notice, there is no fusion between vesicular membrane and cell membrane, network of intracellular canaliculi (9), rough endoplasmic reticulum (10), apical microvilli (red-arrow heads in b), closed non-luminal basal cells (11), pyramidal microvillar cells (12). Inset in 5c showing the apical microvilli of pyramidal microvillar cells.

mitochondria, rough endoplasmic reticulum, free ribosomes (Fig. 6a), whereas, the pyramidal microvillar cells had some smooth endoplasmic reticulum of vesicular appearance (Fig. 6b). This configuration, cells and cell characteristics were similar in all examined animals.

The alveolar portion was lined with columnar microvillar cells that contained an extensive network of intracellular canaliculi, and heterogenous secretory vesicles (Fig. 7).

The ultrastructural features of the glandular microvillar cells of the NSI subepithelial gland were summarized in Fig. 8.

4. Discussion

The present study revealed the presence of a specialized NSI subepithelial gland of unique anatomical structure. Interestingly, there was no duct system, except for a wide intraepithelial duct or canal. This duct was opened directly onto the surface, and continued distally as an alveolar secretory portion with wide lumen. Such glandular architecture is slightly similar to that of the olfactory glands of Bowman's, but with two important differences. Firstly, Bowman's glands' main body, usually found in the form

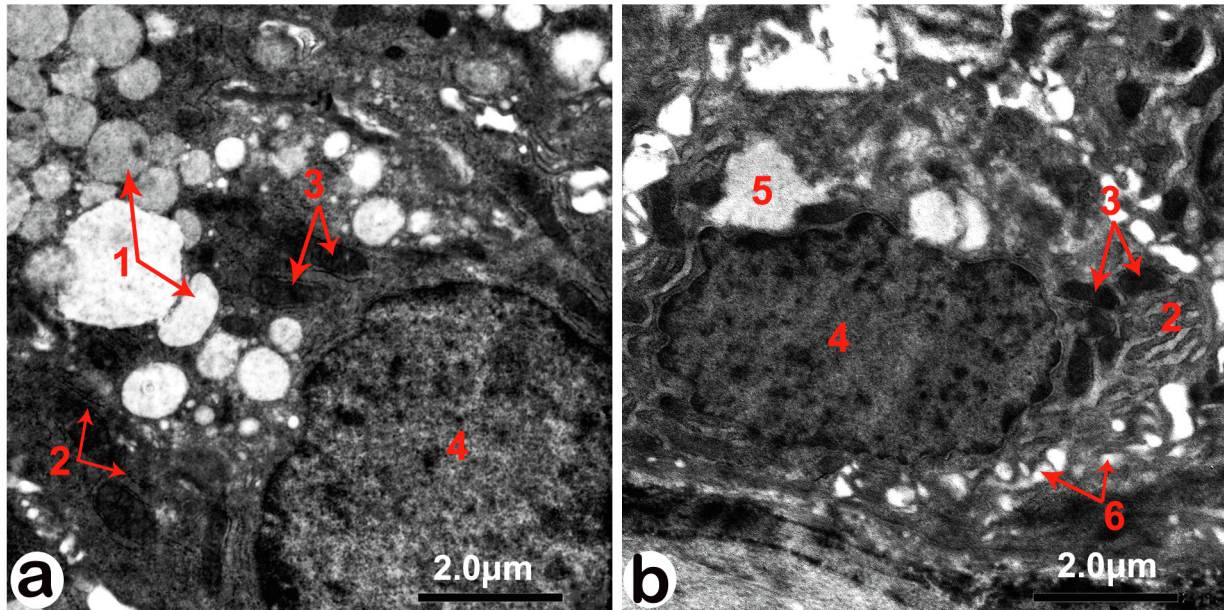


Fig. 6. Ultrathin section of the pre-nuclear region of the columnar (a) and pyramidal (b) microvillar cells, X 4000, showing heterogeneous secretory vesicles (1), rough endoplasmic reticulum (2), mitochondria (3), nucleus (4), smooth endoplasmic reticulum in the form of vesicles (5), intracellular canaliculi (6). Notice, the variable density of the secretory vesicles, the vesicular profile of smooth endoplasmic reticulum, abundant free ribosomes (electron dense dots in the cytoplasm), and the abundant electron-dense mitochondria are characteristic for the APUD cells.

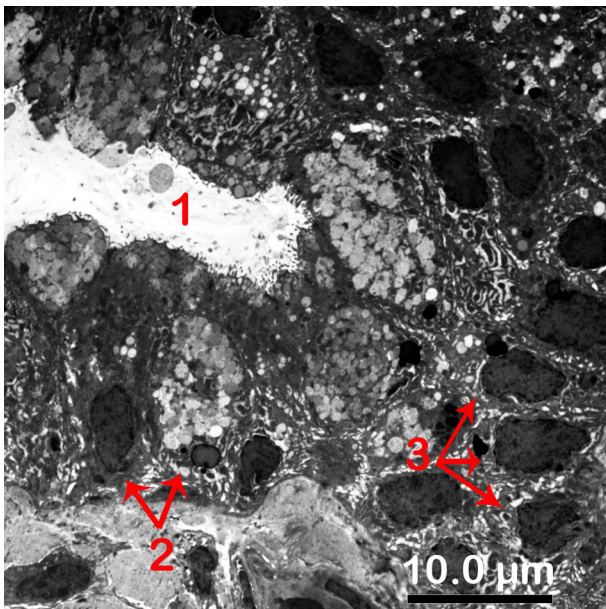


Fig. 7. Ultrathin section of the alveolar portion of the subepithelial gland, X 800, showing lumen of alveoli (1), columnar microvillar cells (2), notice, some cells were sectioned tangentially (3).

of narrow-lumened bulbous acini (Getchell and Getchell, 1992; Fawcett and Jensh, 1997), while NSI gland has alveolar ones. The secretory portion could be described as an alveolus because the lumen is wider than the height of secretory cells (Maynard and Downes, 2019). These anatomical features make the glands look more like ampullae or epithelial pits than true excretory glands. Secondly, the glandular cells at TEM level, showed heterogeneous secretory vesicles of variable electron density, which are characteristic for the APUD cells (Langley, 1994; Crivellato et al., 2003). Moreover, most cells were captured in fixative while they were

undergoing piecemeal degranulation, which is a complex mode of excretion, common for neuroendocrine cells (Langley, 1994; Crivellato et al., 2005).

Notably, the secretory portion was in close contact with numerous capillaries. This may indicate endocrine-paracrine and endocrine-exocrine mode of secretion, described in APUD cells (Pan et al., 2000). As consequence of this outline, we discuss here the above-mentioned information, with special reference to the possible functions.

In contrast to the airway glands, which have a grape like branching tubuloacinar structure, with a non-ciliated collecting duct and a single terminal duct (Widdicombe and Wine, 2015), the NSI subepithelial glands were epithelial pits that open to the exterior through a single intraepithelial duct. This architecture was clearly demonstrated in the sagittal histological sections, which indicates that the glandular units were arranged in imaginary lines, parallel to the facial midline.

Importantly, the NSI glandular cells reacted slightly only with PAS stain, and showed no affinity to the mucus substance stain; Alcian blue, at pH 2.5. However, some cellular clusters within the alveolar lumen positively reacted with Alcian & PAS, indicating the presence of neutral mucins.

Unlike the olfactory glands of Bowman's, which are either entirely or partly mucus (Getchell and Getchell, 1992), and the mucoserous airway glands (Banks, 1993), the NSI subepithelial glands were lined with a series of pale-stained columnar to pyramidal microvillar cells that contained cytoplasmic secretory vesicles, which appeared only with toluidine blue. These observations are suggestive for peptides and amines' synthesis and storage (Langley, 1994). The TEM examinations confirmed this information, where the ultrastructural features of the glandular microvillar cells were typical to those of APUD cells mentioned by Pearse (1969). These features were including abundant rough endoplasmic reticulum and free ribosomes, smooth endoplasmic reticulum in the form of vesicles, electron dense mitochondria and membrane bound vesicles of varying density. Notably, the glandular microvillar cells had the structures required for protein

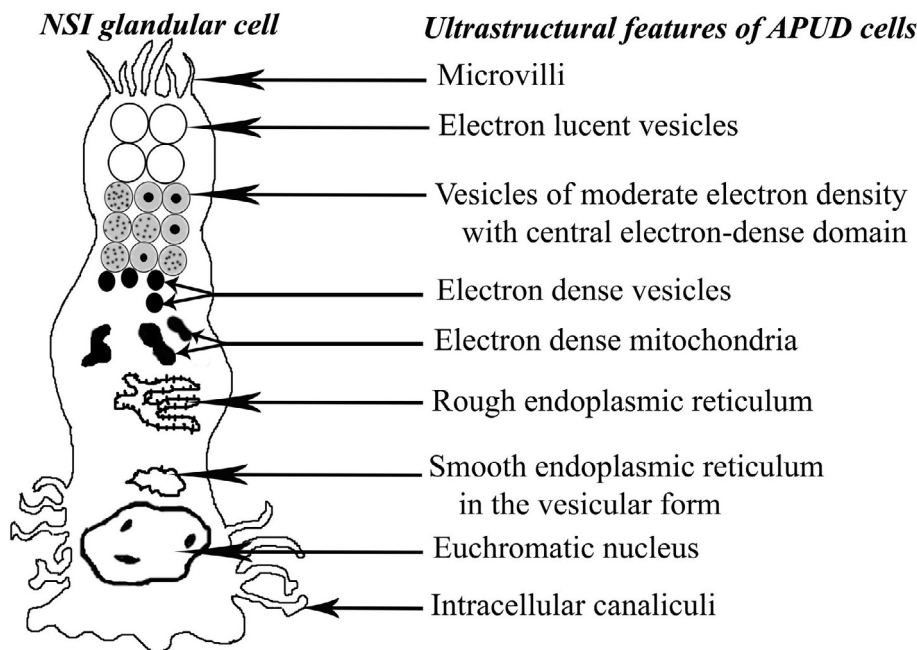


Fig. 8. A schematic representation showing the ultrastructural features of the glandular microvillar cells of the subepithelial gland of the nasal septal island (NSI) in dromedary camels.

synthesis, including euchromatic nuclei, rough endoplasmic reticulum and an extensive network of intracellular canaliculi (Fawcett and Jensch, 1997).

In addition to the protein synthesis structures, these cells also contained secretory vesicles characteristic for catecholamines. Toluidine blue stain was useful in identification and distribution of these neurosecretory vesicles. As a role, catecholamines such as adrenaline and noradrenaline react chemically with glutaraldehyde forming a substrate typically, stained purple or Green (β metachromatic) for adrenaline and noradrenaline, respectively (Honoré, 1971). This glutaraldehyde-toluidine blue method is the same used for semithin preparations, where typical catecholamine containing vesicles (purple-colored) were observed. The colocalities of peptides and catecholamine is the hallmark of APUD cells (Fujita, 1983).

Together, the NSI subepithelial glands have a unique anatomical structure. The anatomical peculiarities of the glandular cells were suggestive for APUD system. These glands are supposed to be specialized for assisting the NSI neuroepithelium in functions related to pain perception (Abo-Ahmed et al., 2021). This possible function depends on the type of proteins, amines and chemical mediators, which the glandular APUD cells are able to produce. The secretory material may play a role in the detection of noxious stimuli, regulation of the immune response as well as inhibition of these noxious cues. However, further studies are required for immunolocalization of peptides and amines related to pain perception in the subepithelial glandular tissue.

5. Conclusion

The subepithelial glands of NSI in dromedaries have unique anatomical features suggestive for APUD system. They have membrane bound secretory vesicles of varying electron density exhibited piecemeal degranulation. As many other APUD cells, they had the structures required for synthesis of a variable number of biologically active peptides, amines and chemical mediators, which may play a role in the detection of noxious stimuli and regulation of the immune response.

Source of funding

The author covers the full cost of this work.

Data availability

The data that support the findings of this study are available from the author upon a reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

All my sincerest thanks to **Prof. Ahmed A. Kassab**, Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, for his encouragement and instructive valuable advice during the preparation of this work.

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