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ARTIGO ORIGINAL/ORIGINAL ARTICLE

O epitélio respiratório em ratos Wistar

Respiratory epithelia in Wistar rats

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RESUMO

A doença vibroacústica é uma patologia sistémica, caracterizada pela proliferação anormal das matrizes extra-celulares e causada pela exposição crónica a ruído de baixa frequência (RBF) (≤500 Hz, incluindo os infra-sons). Neste

ABSTRACT

Morphofunctional changes of respiratory epithelia became the object of intense study in Wistar rats after previous research showed that occupationally-simulated exposure to low frequency noise (≤500 Hz, including infrasound) induced irrever-

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contexto, nos estudos conduzidos em ratos Wistar expostos a RBF, na tentativa de reproduzir a patologia observada em trabalhadores expostos a esta noxa, observaram-se aspectos não descritos. Urge assim esclarecer os aspectos normais em populações de ratos Wistar não expostos. Dez ratos Wistar, tratados de acordo com a norma 86/609/CE, foram mantidos em silêncio até à idade de 3,5 meses e então sacrificados tendo sido colhidos fragmentos de traqueia e brônquios para microscopia óptica e electrónica de varrimento (MEV) e de transmissão (MET). As células em escova (CE) que se observam frequentemente em MET são menos visíveis em MEV, devido aos cílios das células vizinhas. São frequentes as estruturas em roseta, constituídas por um anel de células secretoras centradas numa CE. Em MET observam-se corpos multivesiculares dentro das CE. Observam-se, tanto em MEV como em MET, numerosas vesículas emanando da membrana plasmática dos cílios e das microvilosidades das CE. Estes dados podem contribuir para a compreensão da função das CE.

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Palavras-chave: ruído de baixa frequência, célula em escova, célula secretora, cílios, microvilosidade, morfologia, microscopia electrónica.

sible lesions in these tissues. Aspects of normal respiratory epithelia in rats are lacking in the literature, and are the object of this report. Ten Wistar rats were kept in silence, fed standard rat food, and had unrestrained access to water (treated in accordance with 86/609/CE). The animals were sacrificed at 3.5 months of age, and respiratory epithelial fragments were excised and prepared for scanning (SEM) and transmission (TEM) electron microscopy. Brush cells (BC) were frequently observed with TEM, but with SEM they were often covered by the cilia of neighbouring cells. BC were always observed at the center of a ring of secretory cells (SC), in a rosetta-shaped formation. In TEM, the microvilli of SC surrounding the BC were uniform, and had the same density and shape in all cells. Multivesicular bodies were identified in areas within the BC. Formation and budding of vesicles from ciliary plasma membranes and from BC microvilli were frequently observed in both TEM and SEM. These data contribute to the understanding of the BC function.

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Keywords: low frequency noise, brush cell, secretory cell, cilia, microvilli, morphology, electron microscopy

INTRODUCTION

The pertinence of this report is related to the non-auditory, noise-induced pathology in which animal models have played a central role. The profound morphological changes of the respiratory epithelia observed in noise-exposed Wistar rats has made it essential to have a detailed description of the normal aspects of this tissue structure.

Non-auditory pathology as a consequence of noise exposure has been studied for decades. However, only within the past 20 years has medical diagnostic technology allowed researchers to gather the full extent of this noise-induced pathology[1]. Low frequency noise (LFN) (≤500 Hz, including infrasound) has been identified as an agent of disease responsible for the development of vibroacoustic disease (VAD)[2-4]. VAD is a whole-body, systemic pathology, fundamentally

characterized by the abnormal production of extra-cellular matrices that has been identified in the respiratory system of both LFN-exposed humans[5] and animals[6].

Pleural effusion in three VAD patients (employed as aircraft technicians) became the object of interest in the late 1980's. In all three, the aetiology was unknown, recovery time was unusually long, and response to medication was slow. In an effort to understand these atypical cases, the respiratory system of LFN-exposed Wistar rats began to be studied in 1992. Indeed, results showed that in LFN-exposed animals, the number of microvilli in the pleural parietal leaflet was statistically significantly reduced[7] and pleural mesothelial cells had lost their phagocytic capability[8]. Simultaneously, the immune system of LFN-exposed rodents was deficient which allowed for more severe lesions caused by the presence of atypical mycobacteria in pleural Kampmeir foci[8]. This LFN-induced damage is believed to partially contribute to the atypical evolution of the 3 cases of pleural effusion in VAD patients.

During the preparatory cutting procedures of the rat pleural fragments, a deeper than desired cut was made, and images of the lung parenchyma were also observed under the electron microscope. Focal fibrosis was identified deep in the lung parenchyma[9]. Lung fibrosis was a 1987 autopsy finding in one of the VAD patients who had suffered an atypical case of pleural effusion[10]. At the time, it was assumed that fibrosis was due to exposure to fumes, dusts and chemical agents, also present in this man's occupational environment. However, among the LFN-exposed animal models there had been no contamination with other such agents, and LFN exposure seemed to be the culprit. Subsequently, using high resolution CT scan, lung fibrosis has since been confirmed in 9 of 22 non-smoker, LFN-exposed workers, 6 of whom were asymptomatic[5]. Air-trapping, and/or interlobular septal thickening and/or ground glass opacity was observed in 8 of 15 asymptomatic patients. The effects of LFN on collagen pathology became more pronounced in studies conducted among lupus-prone mice strain and exposed to LFN[11].

Animal models have, thus, played a central role in the research on VAD-associated respiratory pathology. In particular, studies of the respiratory system of Wistar rats exposed to LFN have been yielding a plethora of information[6]. However, there is a lack of studies regarding the normal respiratory epithelia in the Wistar rat. Given the previously reported LFN-induced morphological changes of the respiratory tract cellular populations[6-9,11-13], it is appropriate to have a more detailed and focal description of the normal respiratory epithelia in the Wistar rat. This is the purpose of the present report.

METHODS

Animals

Ten, age-matched Wistar rats were kept in silence, fed standard rat food, and had unrestrained access to water. All animals were obtained from a local breeder (Gulbenkian Institute of Science, Oeiras, Portugal), and treated in accordance with the applicable legislation (86/609/EC). The animals were sacrificed at 3.5 months of age. Fragments of the respiratory system were excised and prepared for scanning (SEM) and transmission (TEM) electron microscopy.

Microscopy

The animals were sacrificed by a lethal intravenous injection of sodium-pentobarbital ($40mg \cdot kg^{-1}$ BW) and the trachea was divided in two along the saggital line. Specimens for light microscopy were formalin-fixed, paraffin-embed-

ded, and stained with hematoxylin-eosin, and fucsin-rhesorcin.

Specimens for electron microscopy were placed in a solution of 3% gluteraldehyde in 0.1 M phosphate buffer, pH 7.2 and then washed with several changes of 5% sucrose in 0.1 M phosphate buffer, pH 7.2, for ultrastructural studies.

Specimens for SEM were dehydrated, critical point-dried and coated with gold-palladium. Examination with the electron microscope (JEOL JSM-35C, Japan) was performed at an accelerating voltage of 15 kV.

For TEM, samples were fixed at room temperature in an aldehyde mixture consisting of 4% paraformaldehyde, 1.25% glutaraldehyde, and 10mM $CaCl_2$ in 0.05 M cacodylate buffer, and pH 7.2. Specimens were washed in buffer, and postfixed in a ferricyanide-reduced osmium solution made up of 1% potassium ferricyanide and 1% osmium tetroxide in distilled water, dehydrated through a graded ethanol series, and embedded in Epon. The samples were sectioned in an ultramicrotome (LKB, Sweden) and the thin sections stained with uranyl acetate and lead citrate. Preparations were then examined with electron microscopy (JEOL 100C, Japan).

RESULTS

With SEM, tufts of cilia of different sizes are observed partially covering both secretory cells (SC) and brush cells (BC).

Multiple rosetta-shaped structures are observable, always formed by a ring of SC centered on a BC (Fig. 1). Depending on the amount of

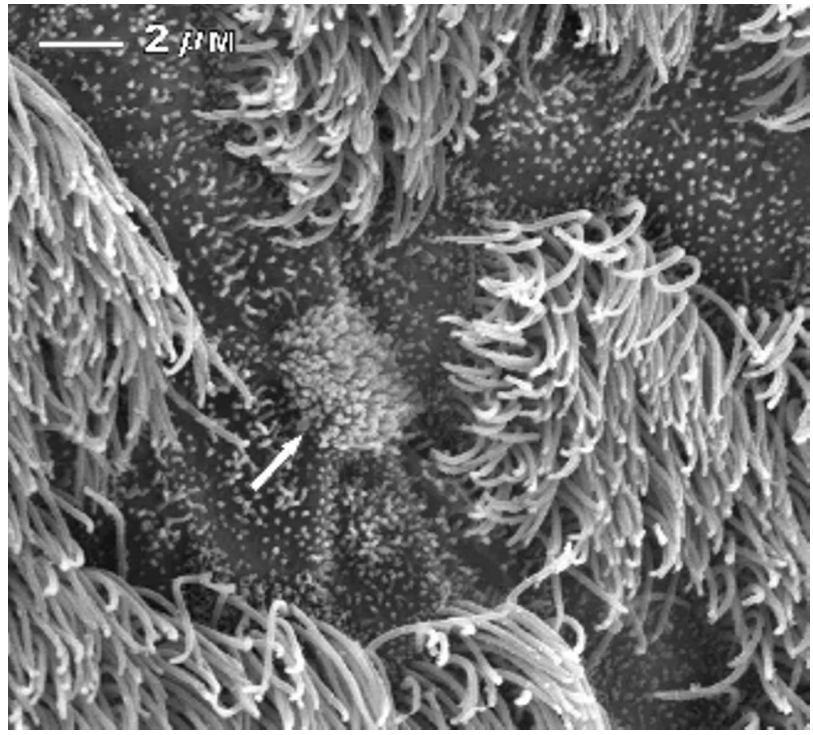

Fig. 1 — SEM of rat tracheal epithelium. Magnification of a rosetta structure, centered on a BC (arrow). BC microvilli are uniformly distributed over the apical surface of the BC, and individual microvilli are promptly indentifiable. SC microvilli are at different lengths. Cilia are long, uniform and in metachrony coordination.

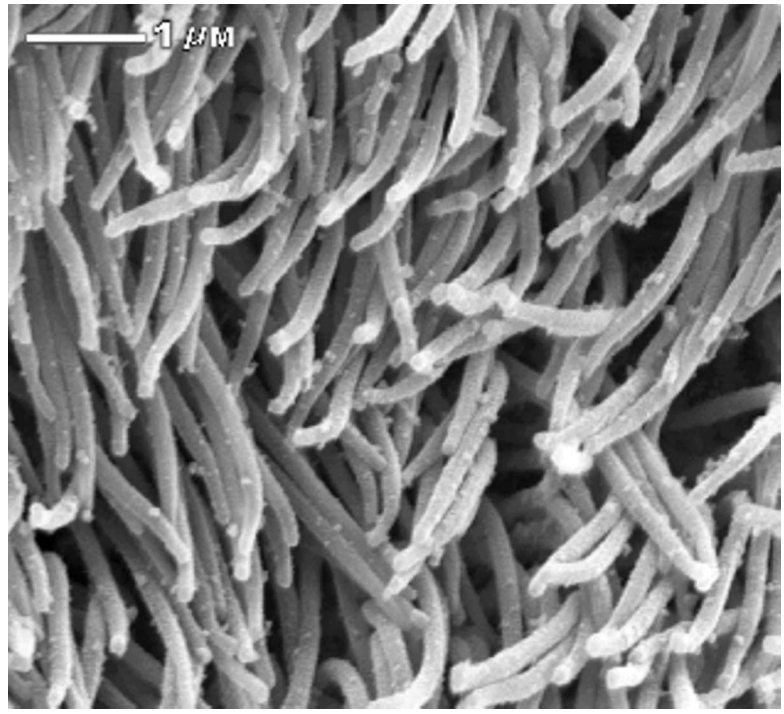

Fig. 2 — SEM of rat tracheal epithelium. Ciliary field where numerous vesicles can be observed along all ciliary axonemes.

neighbouring cilia, BC were more visible in some areas than in others, but they were always observed at the center of rosetta-shaped structures. SC are frequently shared by more than one rosetta structure. SC microvilli are all at different lengths, indicating different stages of the life cycle. BC microvilli are uniformly distributed and cover the entire surface of the BC that is open to the lumen (Fig.1).

Ciliary cells were observed with numerous vesicles attached to the axonemes (Fig. 2). In TEM, in both cross-sectional and longitudinal cuts, vesicles were frequently observed budding from the plasma membrane cilia, in various stages, until the release of the vesicle into the periciliary space. These vesicles are observable in all TEM micrographs, and in SEM micrographs of larger amplification. Numerous macrophages were identified.

In TEM, the relative position of BC and SC corroborate the rosetta-shaped unit seen in SEM (Figs. 3,4). BC microvilli can be seen protruding into the lumen. Vesicles are very frequenty observed budding from BC microvilli. Deep inside the BC body, numerous mitochondria and cytoskeletal structures are visible. Many interdigitations connect the BC to neighbouring cells, forming impressive anchorage mechanisms. The shape of the BC was observed to be related to its age: young BC were cylindrical (Fig. 3) while older cells were pyriform (Fig.4).

The organization of a young BC was partially identified. There seem to be well-defined areas within the young BC body: only the lower half contains multivesicular bodies, interdigitations from the BC to neighbouring cells do not occur uniformly and are mostly close to the area where there multivesicular bodies are present. Other,

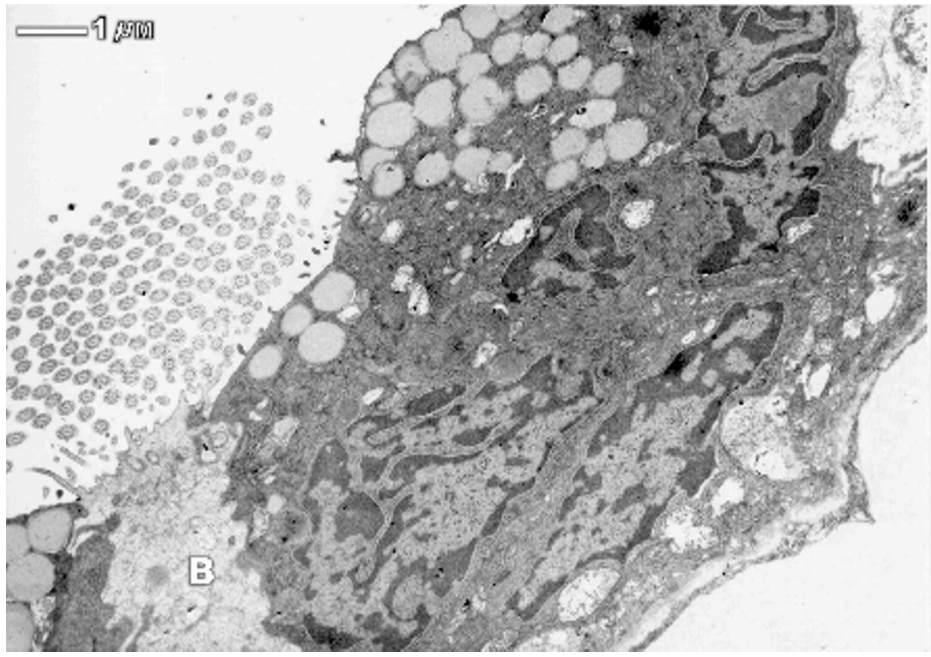

Fig. 3 — TEM of rat tracheal epithelium. A BC (B) sided by two SC. Secretory vesicles are visible within both SC. BC microvilli are protruding into the lumen where cross-sections of cilia can be observed overhanging the brush cell.

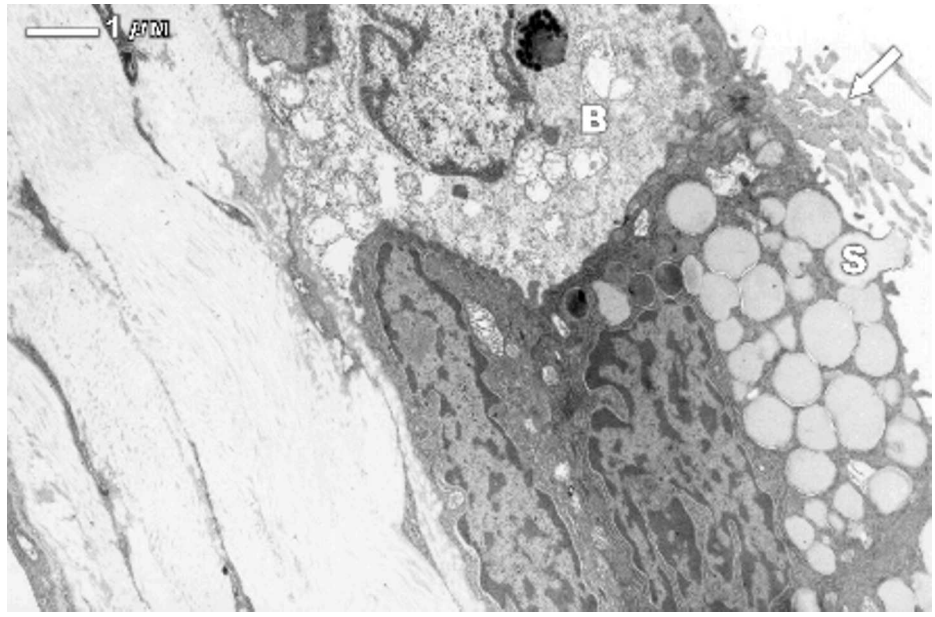

Fig. 5 — TEM of rat tracheal epithelium. A BC (B) sided by a SC where one of its vesicles is releasing into the airspace (S). BC microvilli (arrow) are visible within the airspace. Within the BC, a multivesicular body is located near the nucleus.

smaller vesicle-like structures, deep in the periciliary liquid, were observed and had a different electron density than that of cilia, but were identical to that of microvilli. These are probably not vesicles but sectioned microvilli, because of the similarity in diameters.

DISCUSSION

BC, also known as tufted or caveolated cells, are unipolar epithelial cells seemingly randomly distributed along the respiratory and digestive mucosa[14]. BC are epithelial cells, distinguishable from other epithelial cells because they are considered to be non-ciliated, non-secretory, and present an apical tuft of thick microvilli that are longer than in the adjacent cells[15].

The results presented herein raise more questions than they answer. Vesicles budding from cilia can be observed in other studies[6,16] however no details of this mechanism are known. Vesicles emanating from BC microvilli have, to the authors' knowledge, never been described. Morphologically, this phenomenon does not seem to be related to exocytosis, but further studies are required to confirm whether or not this feature is associated with secretion. Regardless of their possible secretion function, they can certainly influence fluid drainage.

The peculiar organization of BC suggests a variety of complex functions for this cell. Distinct areas within the BC cell body, where the amount of interdigitations seems to be closely associated with the presence of multivesicular bodies, is an important feature that should be considered in future studies. The nature of these multivesicular bodies has recently been associated with neuropeptides[17,18], and continue to be the object of investigation.

In conclusion, the consistency of rosetta-shaped structures formed by a ring of SC centered on a BC, the budding of vesicles from BC microvilli, and the existence of an area containing mutlivesicular bodies that are neuropeptide in nature, strongly suggests that the respiratory tract BC may be responsible for a variety of functions that have yet to be fully determined.

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