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ARTIGO ORIGINAL/ORIGINAL ARTICLE

O epitélio respiratório em ratos Wistar após 48 horas de exposição contínua ao ruído de baixa frequência

Respiratory epithelia in Wistar rats after 48 hours of continuous exposure to low frequency noise

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RESUMO

Em estudos anteriores, demonstrou-se que a exposição crónica de ratos Wistar a ruído de baixa frequência (RBF) provoca o estabelecimento de lesões definitivas no epitélio respiratório. A existência de possíveis períodos refractários

ABSTRACT

Previous studies show that exposure to low frequency noise (LFN) (≤500 Hz, including infrasound) produces irreversible lesions in Wistar rat respiratory epithelia. Recovery periods for LFN-induced lesions have thus become an object

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tornou-se matéria de grande interesse. Neste estudo avaliam-se as alterações do epitélio respiratório de ratos Wistar após uma exposição contínua a RBF. Doze ratos foram expostos a RBF contínuo durante 48 horas. Dez ratos de controlo, do mesmo grupo etário, foram mantidos nas mesmas condições mas em silêncio. Os animais foram tratados de acordo com a norma 86/609//CE. Após a exposição, 2 ratos foram imediatamente sacrificados e grupos de 2 foram sucessivamente sacrificados, respectivamente, após 6, 12, 24, e 48 horas e sete dias em silêncio. Fragmentos do epitélio respiratório foram processados para microscopia óptica electrónica de varrimento. Seis horas após o termo da exposição, era visível tumefação intensa e irregular das células e eram evidentes estruturas em roseta contendo células em escova rodeadas por células secretoras. Os cílios apresentavam-se alterados e as microvilosidades das células em escova estavam agrupadas e perdiam a distribuição uniforme típica e observada nos animais de controlo. Estes aspectos observavam-se nas horas seguintes, embora diminuindo gradualmente de intensidade. Sete dias após o termo da exposição, tanto os ratos de controlo como os ratos expostos e mantidos em silêncio apresentavam epitélios com características não distinguíveis. As lesões epiteliais do aparelho respiratório provocadas pela exposição ao RBF são reversíveis se forem respeitados períodos de recuperação.

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Palavras-chave: ruído de baixa frequência, morfologia, célula em escova, célula secretora, célula ciliada, microvilosidade, doença vibroacústica, tumefação celular, edema.

of interest. Changes in the respiratory epithelia of Wistar rats after continuous short-term exposure to LFN are described. Twelve rats were exposed to continuous LFN for 48 hrs, and 10 age-matched rats were kept in silence. Animals were treated in accordance with 86/609/CE. After exposure ceased, two rodents were sacrificed immediately, and another two after 6, 12, 24, 48 hrs, and 7 days of post-exposure silence. Respiratory epithelial fragments were prepared for light and scanning/transmission electron microscopy. Six hours after exposure, intense and irregular cellular tumefaction was visible and rosetta structures, formed by secretory cells (SC) centered on a brush cell (BC), were identifiable. Cilia were shorter and shaggy. BC microvilli tended to group, losing the uniform distribution seen in controls. Twelve hours after exposure, cell balooning was still present, BC shape was highly irregular and microvilli were grouped. SC microvilli were still shorter than controls. Seven days after exposure, controls and exposed were indistinguishable. LFN-induced epithelial lesions seem to be reversible if recovery periods are respected.

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Key-words: low frequency noise, morphology, brush cell, secretory cell, ciliated cell, microvilli, vibroacoustic disease, tumefaction, cell ballooning, edema

INTRODUCTION

Low frequency noise (LFN) (≤500 Hz, including infrasound) is an agent of disease[1-3], and the respiratory system is a major target for this acoustic stressor[1,4-7]. This has been the motivation for several studies where the respiratory system of LFN-exposed animal models were the object of investigation.

Previous animal studies have demonstrated

that long-term LFN exposure, administered in an occupationally-simulated schedule (8 hours/day, 5 days/week, weekends in silence), had a dramatic effect upon the respiratory tract, such as inducing focal fibrosis of the lung parenchyma[8], diffuse subepithelial fibrosis in the trachea[9], impairment of the phagocytic ability of pleural mesothelial cells, and reduction of the number of microvilli in the pleural parietal leaflet[10]. Cilia were found to be shaggy, sheared or necrotic, cilliary fields were depleted, brush-cell microvilli became fused and squamous metaplasia was observed after 4000 cumulative hours of LFN exposure[11].

Permissible exposure levels and possible recovery periods for lfn exposure have not yet been identified. it is clearly pertinent and highly relevant to investigate the immediate effects of LFN exposure, in order to determine what an appropriate recovery period might be for workers who must remain in LFN environments.

Therefore, the goal of this study is to gain insight into the possible recovery time required by the Wistar rat respiratory epithelia to recuperate from a 48-hrs, continuous exposure to LFN.

METHODS

Noise exposure

A sound signal was generated by an analog noise generator, amplified and frequency filtered. Fig. 1 shows the overall linear and A-weighted noise levels, as well as the spectral analysis of the excitation signal collected at the position near the rat test group inside the chamber. This noise was analyzed by a digital real time analyzer (B&K 2144, Denmark). In this experiment the sound energy was highly concentrated in the lower frequency bands due to the influence of the low-pass filter. In the frequency bands ranging from 50 Hz to 500 Hz the noise levels exceeded 90dB. The overall levels were registered above 109dB, with the A-weighted levels being around 98dB (A)

Animals

Twelve male Wistar rats were exposed to continuous LFN for 48 hrs, and 10 age-matched rodents were kept in equal conditions but in silence. They were 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 were treated in accordance with the European Community Ethics Committee guidelines and regulations for the use of experimental animals (86/609/CE). After the 48-hour exposure ceased, two rodents were sacrificed immediately, and another two after 6, 12, 24, and 48 hours, and 7 days of post-exposure silence.

Microscopy

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

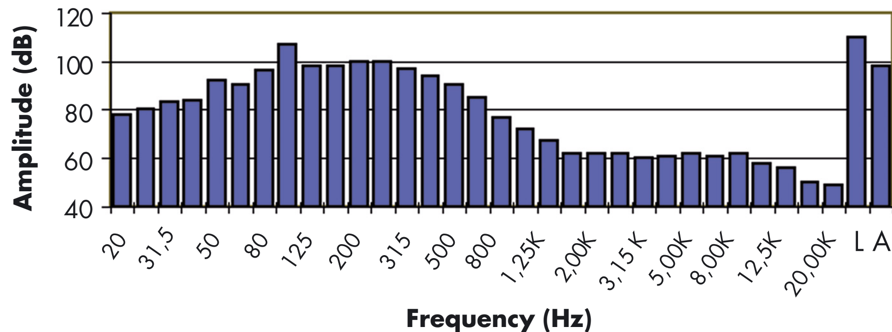

Fig. 1 — Frequency distribution of rat LFN exposure. Ave. Levels: 109 dB_{Lin} (L), 98 dBA (A).

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

Right after exposure, light microscopy revealed discrete hyperemia and edema in all exposed specimens. Leucocytes were rare and there was no predominance of any one type. As post-exposure time progressed, hyperemia and edema became less and less evident. After seven days of post-exposure silence, the epithelial morphology was indistinguishable from that of controls. No vascular lesions were identified.

Figure 2 shows an amplified rosetta structure in a control rat, where each individual microvillus of the BC is clearly visible, and the uniformity of BC microvilli distribution is apparent.

The damage to the respiratory epithelial landscapes observed right after exposure was similar to that observed 6 hours after exposure. Cellular tumefaction was very visible and occured over the entire epithelia, inducing visible swelling of the cells. Orientation and exuberance of cilia was decreased due to ciliar wilting. No sheared cilia were observed. BC microvilli lost their uniform distribution and SC microvilli became shorter. The rosetta structure was conserved and readily visible. Cell ballooning caused distortions in the rosetta structures, and was responsible for the formation of deep valleys at the intercellular junctions. In both control and exposed, the microvilli of the SC forming the rosetta were at different stages of growth, indicating that the SC forming the rosetta have different life-cycles.

Twelve hours after exposure, SC microvilli, in various stages of life cycles, were longer than those in the 6-hour group of rats, but still shorter than in controls (Fig. 3). Cellular tumefaction was still present, and was still the main contributor to the distortion of cell shapes and formation of

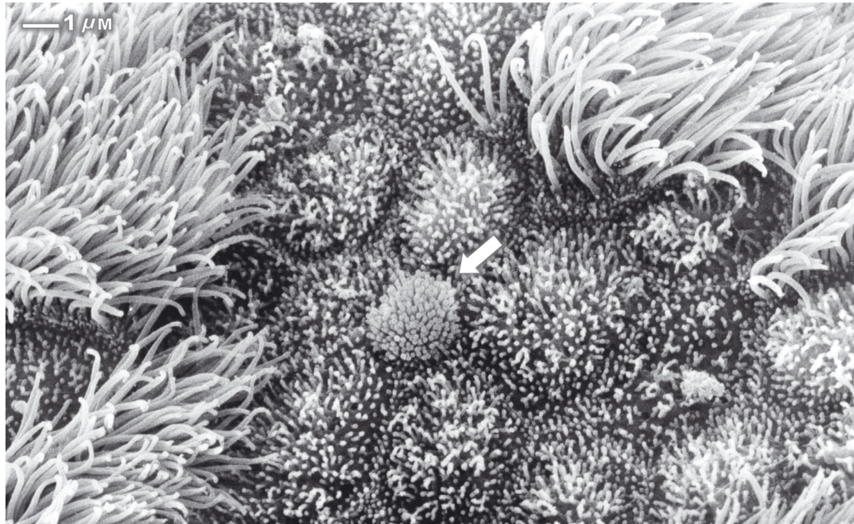

Fig. 2 — SEM of control rat tracheal epithelium. Magnification of a rosetta structure, centered on a BC (arrow). BC microvilli are uniformly distributed over the apical surface of the BC, and individual microvilli are promptly indentifiable. SC microvilli are at different lengths. Cilia are long, uniform and in metachrony coordination.

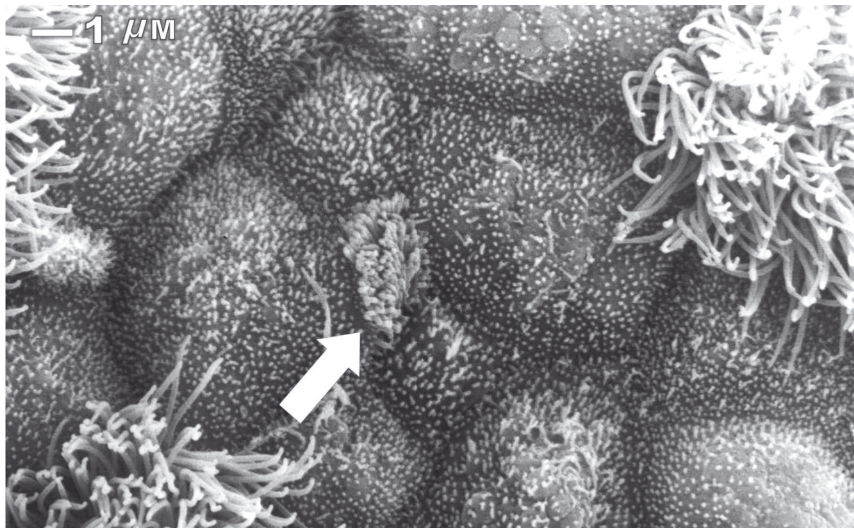

Fig. 3 — SEM of exposed rat tracheal epithelium + 12 hours of silence. Cellular tumefaction is still present distorting the shape of the rosetta structure. The shape of the BC (arrow) and SC are irregular. BC microvilli are irregularly grouped. SC are still at different life cycles (different microvilli sizes and distribution) but the turnover rate seems to be accelerated. Cilia are not exuberant and seem to have lost some of their metachrony coordination. Shaggy cilia are visible. Cell borders are delimited by deep valleys.

intercellular deep valleys. Rosetta structures, centered on a BC, were easily observable. BC microvilli became more evenly distributed over the apical surface, but stilled lacked the overall uniform distribution seen in controls. Cilia had not fully recovered and were still wilted and shaggy. Few differences were observed between the 24- and 48-hour groups. BC microvilli were "blossoming", and were visibly returning to the even distribution seen in non-exposed rats. SC microvilli continued their recovery process, growing longer and becoming more similar to controls. Rosetta structures were readily visible, centered on a BC and surrounded by SC. Cellular tumefaction was still present but to a much lesser extent than in the 6 and 12 hour groups, thus greatly reducing the valleys at the intercellular junctions and the swelling of SC and BC. Cilliary structures were also recuperating well. Seven days after exposure, no hyperemia or cell ballooning could be observed, and the epithelial landscape of the exposed rats was indistinguishable from that of controls.

In TEM, the differences between control and exposed animals were discrete, although the BC of the exposed population exhibited an increased number of electron-dense multivesicular bodies. The cylindrical shape of the BC was practically unaltered in the exposed rats, growing pyriform with age, as in the controls. Vesicles were observed to be budding from cilia and BC microvilli.

DISCUSSION

The morphological changes observed in rat respiratory epithelium after a 48-hour continuous exposure to LFN indicate that this tissue structure is a target for this acoustic stressor. The mechanisms through which trauma occurs are still unidentified. Cell ballooning has also been observed in the kidney of rodents exposed to LFN and is, therefore, considered a non-specific response of the respiratory tissue (in press). Seven days after the 48-hour LFN exposure, the morphological changes observed are indistinguishable from the controls. However, the number of BC microvilli was still less than in controls. The minimum time required for complete reversibility of trauma, has not yet been determined.

There are three features that are constant in all micrographs: a) rosetta-shaped structures formed by a BC surrounded by SC in different stages of life cycles; b) vesicles budding from cilia and BC microvilli, as previously reported[11]; and c) multivesicular bodies that have already been associated with neuropeptides[12,13]. The function of the BC in the respiratory system is, as yet, unknown. BC of the digestive system are known to have a neuroendocrine function, however the same cannot yet be said for the respiratory BC[14,15]. Rosetta structures in epithelial tissue are, to the authors' knowledge, not described in the literature. Clearly, future studies will continue to focus on this structure and the relevance of its changes in response to LFN.

LFN is a non-legislated agent of disease, ubiquitous in modern society, and that causes cumulative damage, regardless of the type of LFN source. At the forefront of our concerns are the individuals who *must* be exposed to continuous large amplitude LFN, such as, pilots, flight attendants, aircraft technicians, urban public transportation operators, disk-jockeys, etc. This report demonstrates that, in rats, LFN-induced damage to the respiratory epithelia can be reversed. This tends to corroborate the observations among VAD patients, whose symptoms disappear when on vacation, sick leave, or retired[1,2]. Other authors have also observed respiratory tract impairment as a consequence of LFN exposure[7,16-18]. Recovery periods should be defined for LFN-exposed workers. However, for that to occur, LFN must first be recognized as an agent of disease.

In conclusion, 48-hour continuous exposure to LFN induces significant morphological

changes in the cellular landscape of rat respiratory epithelia which, given the appropriate recovery period in silence, seem to be reversible.

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