Pathomorphological Peculiarities of Damage to Hair Cells of the Organ of Corti in Experimental Sensorineural Hearing Loss

S. G. Zhuravskii, V. G. Borodulin, V. V. Tomson, and A. I. Lopotko

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Pathomorphology of the organ of Corti was studied on models of acute and chronic sensorineural damage to the acoustic analyzer. Peculiarities of hair cell degeneration, necrosis, and apoptosis in the organ were studied by light and scanning electron microscopy. The type of pathomorphological substrate in abnormalities of the organ of Corti depends on the intensity of the destructive exposure, but not on the nature of otopathological factors.

Key Words: *organ of Corti; hair cell degeneration, necrosis, apoptosis; experimental models of sensorineural hearing loss*

Morphological aspect of hearing loss due to receptor insufficiency is poorly studied. We found no reports characterizing the pathomorphological substrate of sensorineural hearing loss (SNHL) in terms of common pathological processes such as degeneration, necrosis, apoptosis, and inflammation. Hair neuroepithelium of the organ of Corti (OC) as a histogenetically isolated structure is characterized by tissues specificity, which is essential for intraorgan manifestations of nonspecific common pathological states of hair cells (HC).

We studied structural characteristics of OC in experimental acute and chronic SNHL.

MATERIALS AND METHODS

The study was carried out on 45 male Wistar rats (250-300 g) without inflammatory changes in the tympanic membranes shown by otoscopy. The auditory function was evaluated by Preyer reflex using a pediatric audiometer and by otoacoustic emission [2,4,5]. Pathomorphology of OC was studied on SNHL models: acute (ototoxic aminoglycoside [5] and acoustic injuries [2]) and chronic (under conditions of chronic renal insufficiency [4]). Membranous cochlea from intact animals served as the control.

Temporal bones and cochlea were isolated and planar OC preparations were made as described previously [1]. Material for photooptic studies was fixed in 7% formalin in phosphate buffer (pH 7.4). Hematoxylin and eosin staining was used for general evaluation of histological preparations. The status of OC HC was evaluated by the visual picture of stained cell nuclei as described previously [4,5]. Connective tissue was visualized using Van-Gieson staining.

The surface of OC was studied under a JSM-35 scanning electron microscope. The material for electron microscopy was fixed in formaldehyde and glutaraldehyde.

All manipulations with experimental animals were carried out in accordance with requirements of Ethics Committee of I. P. Pavlov Medical University based on the Helsinki Declaration.

RESULTS

Scanning electron microscopy showed that the surface of OC from intact animals presented as rows

Laboratory of Hearing and Speech, Laboratory of Pathomorphology, I. P. Pavlov State Medical University, St. Petersburg. ***Address for correspondence:*** sjour@spmu.rssi.ru. S. G. Zhuravskii

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of monomorphic stereociliary bundles, stretched along the cochlear helix (W-shaped bundles of internal HC stereocilia closer to the central axis of the cochlea, V-shaped bundles of external HC stereocilia outside; Fig. 1, *a*). The structure of OC was identical at all curls of the cochlea, but there were qualitative differences in the volume of cell nuclei and structure of the stereociliary system.

One of the first changes in OC HC under conditions of experimental disease [2,4,5] is ectopic location of the nucleoli at the edge of the nuclear membrane and moderate enlargement of the nuclear volume (Fig. 2, *b*, *c*; "cell nucleus pulsation phenomenon") [7]. This state is reversible, develops under the influence of atraumatic factors, and is etiologically nonspecific [6]. Similar changes in the nuclei were described for sensory cells of the retina and spinal motoneurons [7].

At the ultrastructural level HC in a state of degeneration were characterized by disorderly stereociliary edge (Fig. 2, *d*) and stereociliary edema (Fig. 2, *a*). The latter was probably caused by inhibition of membranous ionic channels as a result of oxidative damage [2].

Long-term exposure or high intensity of destructive factors leads to irreversible changes. Hair neuroepithelial necrosis manifests in degeneration of sensory and supporting cells. HC necrosis is well studied, while the data on apoptosis are scanty.

Light microscopy without immunohistochemical staining verifies apoptosis at final stages of its development (during the degradation phase). Con-

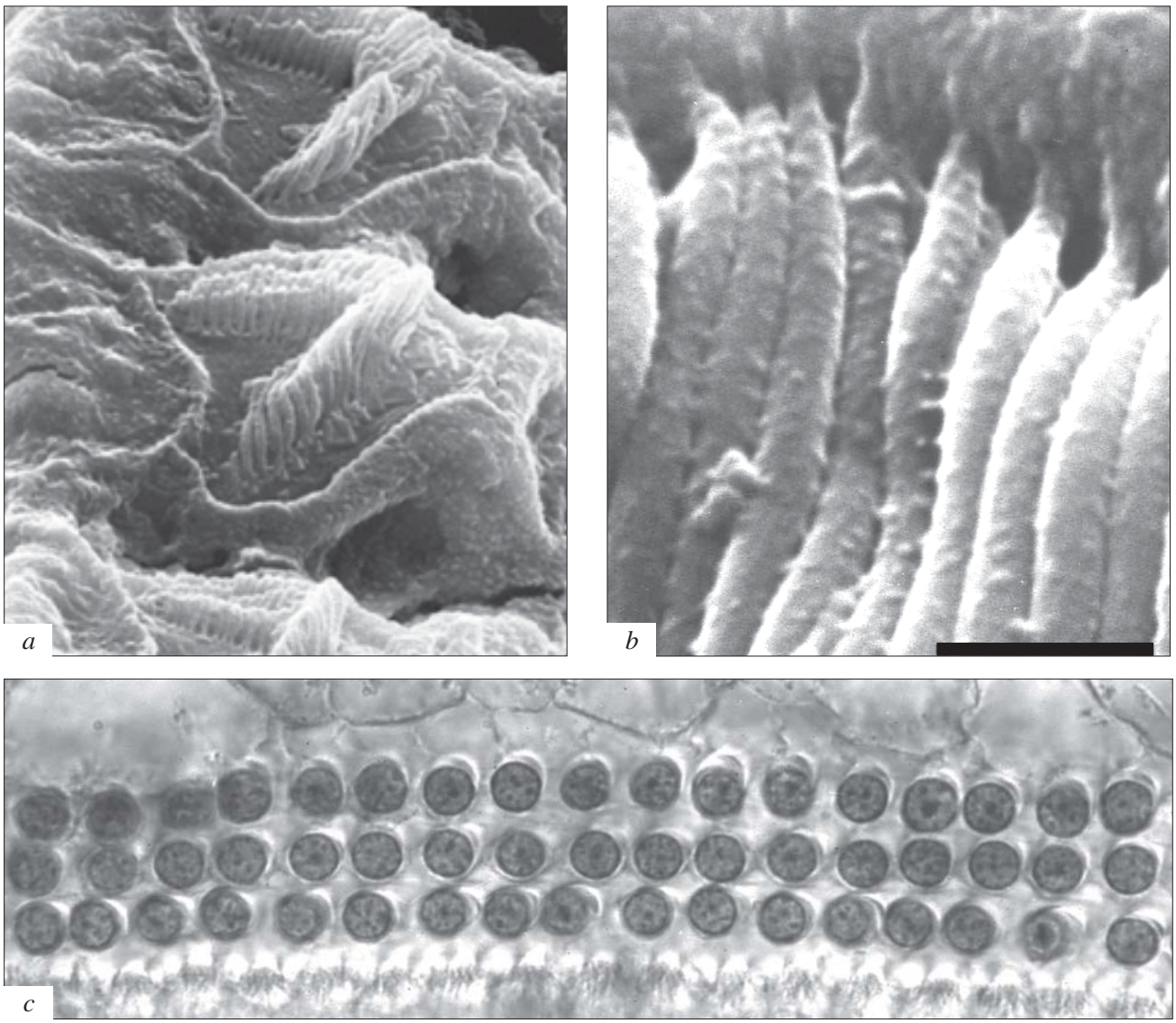

Fig. 1. Organ of Corti from an intact rat. *a*) ultrastructure of the surface of external hair cells (HC), ×6000; *b*) ultrastructure of stereociliary system of internal HC, ×20 000; *c*) organ of Corti in the zone of external HC. Planar preparation. L. Einarson staining, ×630.

densed chromatin of apoptotic HC (apoptotic bodies) is seen as optically dense hyperchromatic material in characteristic circles or semicircles (Fig. 3, *a*). Bubbles on cell surface (blebbing phenomenon), an indirect sign of early apoptosis, can be seen on OC surface. The biological significance of this phenomenon is concentration of the cell material by removal of water (Fig. 3, *b*).

No fragmentation of condensed material was seen among HC apoptotic figures. This can serve as a pathognomonic sign for apoptosis of sensory OC cells.

High phagocytic activity of supporting epitheliocytes [11] was noted (Fig. 3, *c*). Results of apoptosis of HC, sites of absent cells in OC neuroepithelial layers, are more often seen on preparations (Fig. 3, *d*, *e*). These changes can be seen after treatment with aminoglycosides, cisplatin, under conditions of chronic renal insufficiency, and chronic noise exposure [2-5,8,9,11,13]. This process is asynchronous in neighboring cells, which fact explains the presence of just solitary apoptosis figures in tissues (in contrast to necrosis).

By the incidence of pathological factors in the etiological structure of SNHL, apoptosis of HC in OC is the primary morphological substrate of hearing insufficiency. Development of aminoglycoside-induced apoptosis depends on the rate of attaining critical concentration of the antibiotic around HC; in acoustic damage to OC apoptosis is the mechanism of HC death during exposure to noise

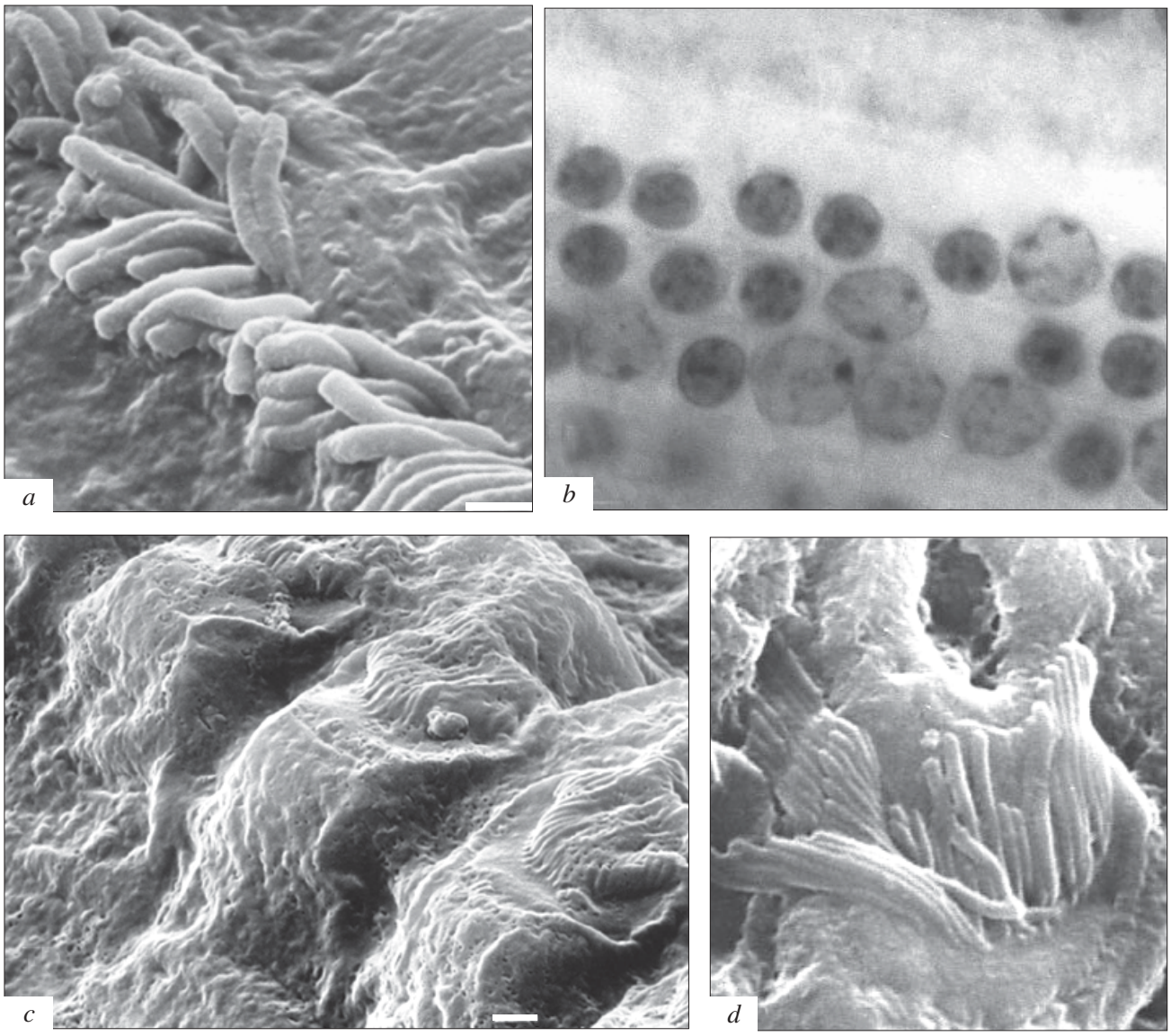

Fig. 2. Rat organ of Corti after acute acoustic trauma (exposure to 5 kHz current, 100 dB for 1 h). *a*) HC stereociliary edema, ×9400; *b*, *c*) HC "nucleus pulsation" phenomenon. Planar preparation. Einarson staining, ×630 (*b*), ×2000 (*c*); *d*) disordered rows of HC stereocilia, ×4800.

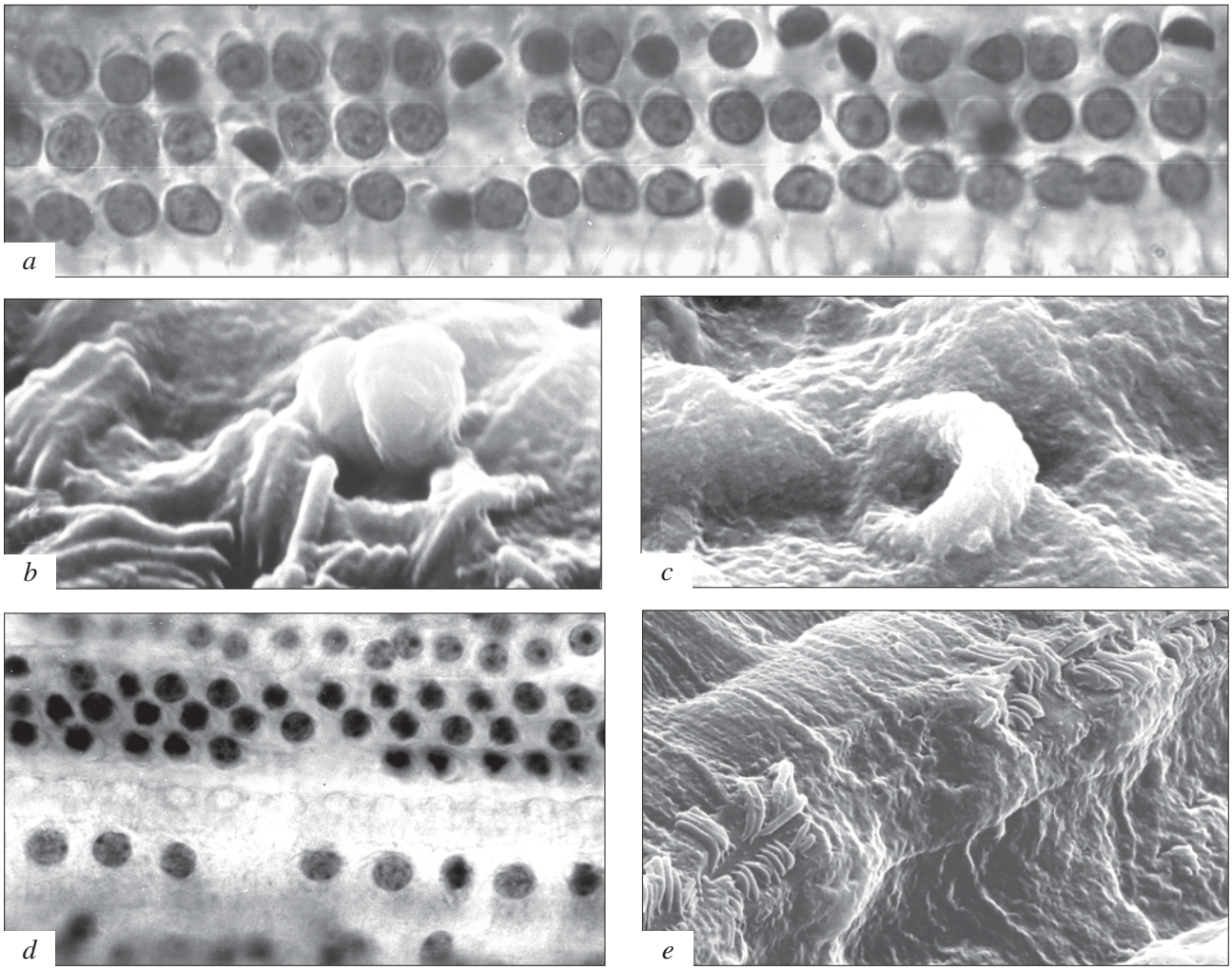

Fig. 3. Rat organ of Corti under conditions of sensorineural hearing loss. *a*) half-moon HC apoptotic bodies. Chronic sensorineural hearing loss under conditions of metabolic disorders in chronic renal insufficiency (month 6 after operation). Staining after L. Einarson, ×630; *b*) formation of bubbles on HC surface after acute acoustic injury (exposure to 5 kHz current, 100 dB for 4 h), ×18 000; *c*) erythrocyte phagocytosed by supporting epitheliocyte. Conditions after acute acoustic injury (exposure to 5 kHz current, 100 dB for 4 h), 18 000; *d*) gaps in row I of external HC and in the row of internal HC of a rat 3 weeks after exposure to an aminoglycoside antibiotic (50 mg/kg kanamycin daily intraperitoneally for 10 days). Staining after L. Einarson, ×630; *e*) "epithelial cicatrix" at the site of dead internal HC (exposure to 5 kHz current, 100 dB for 4 h), ×8600.

and in case of delayed death of cochlear epithelium [8]. Endogenous conditions of internal ear cell apoptosis emerge during involution processes [14], metabolic disorders [3,4].

Alteration of not only HC, but of other structures of the membranous cochlea leads to replacement of the entire helical channel of the cochlea with connective tissue followed by its ossification. Apoptotic death of some HC is followed by compensatory hypertrophy of Deiters' supporting cells (Fig. 3, *e*) with the formation of a permanent "epithelial cicatrix" [11], due to which the intact surface of the reticular plate is retained and further structural and functional disorders of acoustic perception at the adjacent OC sites with intact HC are prevented.

Hence, histogenetic isolation, peculiarities of the microstructure and homeostasis of the receptor component of the acoustic analyzer determine specific pathological changes in the hair neuroepithelial cells.

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