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THE PRE- AND POST-SOMATIC SEGMENTS OF THE HUMAN TYPE I SPIRAL GANGLION NEURONS – STRUCTURAL AND FUNCTIONAL CONSIDERATIONS RELATED TO COCHLEAR IMPLANTATION

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Abstract—Human auditory nerve afferents consist of two separate systems; one is represented by the large type I cells innervating the inner hair cells and the other one by the small type II cells innervating the outer hair cells. Type I spiral ganglion neurons (SGNs) constitute 96% of the afferent nerve population and, in contrast to other mammals, their soma and pre- and post-somatic segments are unmyelinated. Type II nerve soma and fibers are unmyelinated. Histopathology and clinical experience imply that human SGNs can persist electrically excitable without dendrites, thus lacking connection to the organ of Corti. The biological background to this phenomenon remains elusive. We analyzed the pre- and post-somatic segments of the type I human SGNs using immunohistochemistry and transmission electron microscopy (TEM) in normal and pathological conditions. These segments were found surrounded by non-myelinated Schwann cells (NMSCs) showing strong intracellular expression of laminin-β2/collagen IV. These cells also bordered the perikaryal entry zone and disclosed surface rugosities outlined by a folded basement membrane (BM) expressing laminin-β2 and collagen IV. It is presumed that human large SGNs are demarcated by three cell categories: (a) myelinated Schwann cells, (b) NMSCs and (c) satellite glial cells (SGCs). Their BMs express laminin-β2/collagen IV and reaches the BM of the sensory epithelium at the habenula perforata. We speculate that the NMSCs protect SGNs from further degeneration following dendrite loss. It may give further explanation why SGNs can persist as electrically excitable monopolar cells even after long-time deafness, a blessing for the deaf treated with cochlear implantation. **© 2014 The Authors. Published by Elsevier Ltd. on behalf of IBRO. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).**

Key words: human cochlea, spiral ganglion neurons, non-myelinated Schwann cells, laminin-β2, collagen IV, immunohistochemistry.

INTRODUCTION

Loss of auditory receptors commonly leads to a retrograde degeneration of the auditory nerve. In man, this process seems slow, incomplete (Nadol et al., 1989; Nadol and Hsu, 1991; Turner et al., 2008) and mostly involves the peripheral process or dendrite. Thus, spiral ganglion neurons (SGNs) may persist without peripheral connections to the sensory organ as monopolar or "amputated" cells with unbroken connections to the brain (Felder et al., 1997; Glueckert et al., 2005; Teufert et al., 2006; Linthicum and Fayad, 2009). There is clinical evidence that these neurons are electrically excitable even after many years of deafness (Liu et al., 2014). Pertinent cochlear implant (CI) results can be

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Abbreviations: AIS, axonal initial segment; BM, basement membrane; CI, cochlear implants; Cx43, connexin 43; ECM, extracellular matrix; EDTA, ethylene-diamine-tetra-acetic acid; IHC, immunohistochemistry; LM, light microscopy; MBP, myelin basic protein; Nav1.6, Na^+-channels; NIHL, noise-induced hearing loss; NMSC, non-myelinated Schwann cell; PBS, phosphate-buffered saline; SEM, scanning electron microscopy; SG, spiral ganglion; SGC, satellite glial cell; SGN, spiral ganglion neuron; TEM, transmission electron microscopy; type I ganglion cells, large afferent neurons innervating inner hair cell; type II ganglion cells, small afferent neurons innervating outer hair cells.

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achieved in patients with even ossified or partly ossified cochleae where the peripheral nerve axons or dendrites can be assumed to be non-existent. Consequently, hearing impaired individuals may exhibit similar auditory profiles with divergent neuropathological changes. An appealing consequence is the current aptitude to re-sprout SGNs under relevant conditions to further improve CI results.

The essential roles played by surrounding satellite glial cells (SGCs) for the protection of neurons in various sensory ganglia have been broadly described (Pannese, 1981; Hanani et al., 2002, 2010; Hanani, 2010). We recently postulated that connexin 43 (Cx43)-mediated gap junction signaling between SGCs might play a key role and explain the unique preservation of auditory neurons following hair cell loss (Liu et al., 2014). In this paper, an additional bordering cell, named non-myelinated Schwann cell (NMSC) is described. It surrounds the pre- and post-somatic initial segments of the human type I SGNs. Its potential role in the consolidation of SGNs following dendrite atrophy is described.

EXPERIMENTAL PROCEDURES

Our study was approved by the Uppsala Ethics Review Board (No. 99398, 22/9 1999, cont., 2003, Dnr. 2013/190). Written information was given to the patient and verbal informed consent was obtained. This procedure was chosen to reduce the stress on the patient and was approved by the ethics review board. No personalized notes were made to reduce traceability and maintain patient confidentiality. The studies adhered to the rules of the Helsinki declaration. Animal presentations are from archival material in Innsbruck.

Fixation and sectioning of human cochlea

Seven cochleae belonging to seven adult patients (two male, five females; ages 40–65 years) with normal pure tone thresholds for their age were dissected out as a whole piece during petroclival meningioma surgery. In the operating room, the cochleae were immediately placed in 4% paraformaldehyde diluted with 0.1 M phosphate-buffered saline (PBS; pH 7.4). After a 24-h fixation, the fixative was replaced with 0.1 M PBS then with 0.1 M ethylene-diamine-tetra-acetic acid (Na-EDTA) solution at pH 7.2 for decalcification. After 4 weeks, the decalcified cochleae were rinsed with PBS. For frozen sections, the cochleae were embedded in Tissue-Tek (OCT Polysciences, Tokyo, Japan), rapidly frozen and sectioned at 8–10 μm using a Leica cryostat microtome. The frozen sections were collected onto gelatin/chrome-alum-coated slides and stored below −70 °C before immunohistochemistry (IHC).

Antibody and IHC

The antibody against laminin-β2 was a monoclonal antibody from the rat (catalog number #05-206, Millipore, Billerica, MA, USA; dilution 1:100). It recognizes and is specific for the β2 chain of laminin. It does not cross-react with other basement membrane (BM) components or fibronectin (FN). The type IV collagen antibody was a goat polyclonal antibody (catalog number AB769, Millipore, Billerica, MA, USA; dilution 1:10). It has less than 10% cross reactivity with collagen types I, II, III, V and VI. Myelin basic protein (MBP) antibody was a polyclonal antibody from rabbit (catalog number #AB980, Millipore, Billerica, MA, USA; dilution 1:100). The antibody against neuron-specific class III beta-tubulin (Tuj1) was a polyclonal antibody (catalog number #04-1049, Millipore, Billerica, MA, USA; dilution 1:200). Another Tubulin antibody was a monoclonal antibody from the mouse (catalog MAB1637, Millipore, Billerica, MA, USA; dilution 1:200). Antibody combination, characteristics and sources are summarized in Table 1 and more information can be found under discussion. IHC procedures on cochlear sections were described in previous publications (Liu et al., 2011, 2012). Briefly, incubation of sections on slides with solution of the antibodies was carried out under humid atmosphere at 4 °C for 20 h. After rinsing with PBS (3 × 5 min), the sections were subsequently incubated with secondary antibodies conjugated to Alexa Fluor 488 and 555 (Molecular Probes, Carlsbad, CA, USA), counter-stained with a nuclear stain DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) for 5 min, rinsed with PBS (3 × 5 min) and mounted with a VECTA SHIELD (Vector Laboratories, Burlingame, CA, USA) mounting medium. The sections used for antibody control were incubated with 2% bovine serum albumin (BSA) omitting the primary antibodies. As the result of the control experiment, there was no visible staining in any structure of the cochleae.

Imaging and photography

Stained sections were investigated with an inverted fluorescence microscope (Nikon TE2000, Nikon Co., Tokyo, Japan) equipped with a spot digital camera with three filters (for emission spectra maxima at 358, 461 and 555 nm). Both microscope and camera are connected to a computer system installed with image software (NIS Element BR-3.2, Nikon) including image merging and a fluorescence intensity analyzer. For laser confocal microscopy, the same microscope was used which is equipped with laser emission and detection system with three different channels. The optical scanning and image-processing tasks were run by the program Nikon EZ-C1 (ver. 3.80) including reconstruction of Z-stack images into projections or 3D images.

Transmission electron microscopy (TEM) analysis

Two human specimens were analyzed at inner ear research laboratories in both Uppsala and Innsbruck. Tissue was rinsed in cacodylate buffer, followed by fixation with 1% osmium tetra-oxide at 4 °C for 4 h. Subsequently the cochleae were decalcified in 0.1 M Na-EDTA, pH 7.4, for 6 weeks. After decalcification, a mid-modiolar section was made and the two halves were then dehydrated in an increasing ethanol series and acetone prior to incubation in a dilution of liquid epoxy

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