Hearing Research 345 (2017) 79–87

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research Paper

Degeneration of auditory nerve fibers in guinea pigs with severe sensorineural hearing loss

Steven Kroon [a], Dyan Ramekers [a, b], Emma M. Smeets [a], Ferry G.J. Hendriksen [a], Sjaak F.L. Klis [a, b], Huib Versnel [a, b, *]

[a] *Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, Room G.02.531, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands*
[b] *Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands*

A R T I C L E I N F O

Article history:
Received 27 June 2016
Received in revised form
7 December 2016
Accepted 6 January 2017
Available online 10 January 2017

Keywords:
Auditory nerve
Spiral ganglion cell
Axon
Degeneration
Hearing loss
Guinea pig

A B S T R A C T

Damage to and loss of the organ of Corti leads to secondary degeneration of the spiral ganglion cell (SGC) somata of the auditory nerve. Extensively examined in animal models, this degeneration process of SGC somata following deafening is well known. However, degeneration of auditory nerve axons, which conduct auditory information towards the brainstem, and its relation to SGC soma degeneration are largely unknown. The consequences of degeneration of the axons are relevant for cochlear implantation, which is applied to a deafened system but depends on the condition of the auditory nerve. We investigated the time sequence of degeneration of myelinated type I axons in deafened guinea pigs. Auditory nerves in six normal-hearing and twelve deafened animals, two, six and fourteen weeks (for each group four) after deafening were histologically analyzed. We developed a semi-automated method for axon counting, which allowed for a relatively large sample size (20% of the total cross-sectional area of the auditory nerve). We observed a substantial loss of auditory nerve area (29%), reduction in axon number (59%) and decrease in axoplasm area (41%) fourteen weeks after deafening compared to normal-hearing controls. The correlation between axonal degeneration and that of the SGC somata in the same cochleas was high, although axonal structures appeared to persist longer than the somata, suggesting a slower degeneration process. In the first two weeks after induction of deafness, the axonal cross-sectional area decreased but the axon number did not. In conclusion, the data strongly suggest that each surviving SGC possesses an axon.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Damage to or loss of inner hair cells (IHC) of the cochlea results in sensorineural hearing loss (SNHL). Secondary to IHC loss, the spiral ganglion cells (SGCs), the axons of which form the auditory nerve, degenerate progressively (Spoendlin, 1971, 1975; Webster and Webster, 1981; Leake and Hradek, 1988; Nadol et al., 1989; Versnel et al., 2007; Glueckert et al., 2008). The extent of degeneration is correlated to the duration of deafness for both humans and animals (Spoendlin, 1975; Fayad and Linthicum, 2006; van Loon et al., 2013). This degeneration process has mostly been described as retrograde, starting with the peripheral processes (Spoendlin, 1975, 1984; Leake and Hradek, 1988). Leake and Hradek (1988) reported first swelling, demyelination and degeneration of the peripheral processes, followed by demyelination and shrinkage of the SGC somata and finally demyelination of the axons and SGC soma loss. The fate of the axons in this degeneration process, however, is not well studied, in spite of its obvious relevance for hearing after cochlear impairment, whether acoustic or with a cochlear implant.

Degeneration of SGC somata and their peripheral processes have been studied extensively in both humans (e.g., Otte et al., 1978;

Abbreviations: 2WD, two weeks after deafening; 6WD, six weeks after deafening; 14WD, fourteen weeks after deafening; AN, auditory nerve; IAM, internal acoustic meatus; IHC, inner hair cell; NH, normal-hearing; SGC, spiral ganglion cell; SNHL, sensorineural hearing loss

* Corresponding author. Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, Room G.02.531, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands.

E-mail addresses: stevenkroon1@gmail.com (S. Kroon), d.ramekers@umcutrecht.nl (D. Ramekers), emmasmeets@gmail.com (E.M. Smeets), f.hendriksen@umcutrecht.nl (F.G.J. Hendriksen), s.klis@umcutrecht.nl (S.F.L. Klis), h.versnel@umcutrecht.nl (H. Versnel).

http://dx.doi.org/10.1016/j.heares.2017.01.005
0378-5955/© 2017 Elsevier B.V. All rights reserved.

Hinojosa and Marion, 1983; Hinojosa et al., 1987; Nadol, 1988) and animals (e.g., Spoendlin, 1984; Leake and Hradek, 1988; Waaijer et al., 2013; Ramekers et al., 2015a). In contrast, quantitative histological studies of auditory nerve axons are scarce. In five studies, the auditory nerve axons of normal-hearing animals were quantified (Boord and Rasmussen, 1958; Gacek and Rasmussen, 1961; Alving and Cowan, 1971; Ehret, 1979; Köppl, 1996). Gacek and Rasmussen (1961) found an average of approximately 24,000 axons in the auditory nerve of normal-hearing guinea pigs. The healthy human auditory nerve consists of approximately 32,000 axons (Felix et al., 1990; Rasmussen, 1940; Spoendlin and Schrott, 1989). In humans with SNHL, the auditory nerve axon number is lower (Ylikoski et al., 1978, 1981; Ylikoski and Savolainen, 1982, 1984; Felix and Hoffmann, 1985; Spoendlin and Schrott, 1989). Degeneration of the auditory nerve includes a decrease in the total cross-sectional area of the auditory nerve and a decrease in number of Schwann cells as observed in deafened guinea pigs (Kong et al., 2010) and a decrease in axonal diameter as observed in humans with SNHL (Spoendlin and Schrott, 1989, 1990). However, the course of degeneration for axons following IHC loss has not been quantified, and it is not known how axonal degeneration relates to the degeneration of the cell body.

Better understanding of the degeneration process in SNHL is important because the number of axons and axonal morphology are likely to influence neural conduction. Furthermore, extensive knowledge of this process could be useful in assessing the human auditory nerve integrity with electrophysiological measurements (e.g., Brown et al., 1990; Botros and Psarros, 2010; Mehraei et al., 2016) or imaging techniques (e.g., Glastonbury et al., 2002; Vos et al., 2015). Thus, the first objective of this study was to develop a reliable and practicable method for quantitative auditory nerve axon analysis. The second objective was to accurately determine the number of axons in the normal-hearing and deafened guinea pig auditory nerve and subsequently compare the extent of auditory nerve degeneration with that of the SGC somata in the same cochleas.

2. Methods

2.1. Animals and experimental design

In this study, 18 adult female albino guinea pigs (strain: Dunkin Hartley; weight: 250–350 g; age: 3–4 weeks) from Harlan Laboratories, Horst, the Netherlands were included. All animals were housed in small groups under standard laboratory conditions (free access to food and water; 12 h light cycle; 21 °C and 60% humidity). Normal hearing was confirmed for all animals with acoustically evoked auditory brainstem responses (ABRs) prior to any experimental treatment. Animals were randomly allocated to one of four experimental treatment groups. One group served as normal-hearing controls (NH, $n = 6$). Animals in the other experimental groups ($n = 4$ per group) were deafened by a co-administration of kanamycin and furosemide. These groups consisted of animals that were deafened two (2WD), six (6WD) or fourteen (14WD) weeks before acute electrophysiological experiments. The animals were sacrificed and processed for histology directly after these electrophysiological experiments. The cochleas of NH, 2WD and 6WD animals were previously used by Ramekers et al. (2014) for the correlation between electrophysiological measures and SGC survival. Animals with an acoustically evoked ABR threshold shift less than 50 dB after ototoxic treatment were excluded from the study, following the criterion of severe hearing loss applied in our previous studies (van Loon et al., 2013; Ramekers et al., 2014, 2015b). All experiments were approved by the Animal Experiments Committee of Utrecht University (before 2015; DEC 2010.I.08.103) or by the Dutch Central Animal Experiments Committee (since 2015; CCD 11500201550).

2.2. Surgical procedures

Deafening was performed by subcutaneously injecting the aminoglycoside antibiotic kanamycin (Sigma-Aldrich, St. Louis, MO, USA; 400 mg/kg), followed by the loop diuretic furosemide (Centrafarm, Etten-Leur, the Netherlands; 100 mg/kg) through an external jugular vein catheter. This deafening procedure, introduced by West et al. (1973), has been shown to eliminate most inner and outer hair cells (Versnel et al., 2007). Actual ABR threshold shifts were 68 dB on average, ranging from 50 to 82 dB, confirming severe to profound hearing loss.

For other experiments, not reported in the present paper, all animals were implanted at the end of each experiment with a four-contact electrode array for recordings of electrically evoked compound action potentials (eCAPs). For detailed descriptions of the methods regarding surgical procedures and eCAP recordings, see van Loon et al. (2013) and Ramekers et al. (2014), respectively.

2.3. Histological analysis

In the present study, only the right cochleas of the animals were used for histological analysis. Intra-labyrinthine cochlear fixation was carried out with 3% glutaraldehyde, 2% formaldehyde, 1% acrolein and 2.5% dimethyl sulfoxide (DMSO) in a 0.08 M sodium cacodylate buffer (pH 7.4). This method for cochlear fixation is described by de Groot et al. (1987). The cochleas were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) in distilled water (pH 7.4), post-fixated in 1% osmiumtetroxide in distilled water, dehydrated in graded ethanol series, cleared with propylene-oxide and embedded in Spurr's low-viscosity resin.

2.3.1. Axonal analysis

Transverse, semi-thin (1 μm) sections of the auditory nerve (Fig. 1A and B) were cut, beginning at 500 μm from the internal acoustic meatus (IAM) and moving with steps of 25 μm to the direction of the cochlea until Scarpa's ganglion was no longer visible. At this level, the auditory nerve is separated from the vestibular nerve. We estimated both the angle and position of the section of the nerve in order to detect possible differences among treatment groups that might influence the axon count. We measured the distance d from the axonal section to the IAM and the angle (α) of the axonal section with the modiolus for all animals (see Fig. 1A). The distance d was estimated by overlaying the midmodiolar section obtained for SGC analysis (see section 2.3.2) with a midmodiolar section from another animal (of approximately the same size) that was not sectioned for axonal analysis. In this section the IAM and modiolus are still present in contrast to sections used for axonal analysis (Fig. 1A). The intact section was rotated in such a way that both modioli exactly overlapped. The average distance d for all groups was 616 μm, with a standard deviation of 101 μm, and did not differ statistically among treatment groups (one-way ANOVA $F_{(3)} = 0.31$, $p = 0.81$). The cutting angle was defined as the angle of the cut for axonal analysis and the modiolus. The average angle was 34.3°, with a standard deviation of 5.4°, and was not statistically different among treatment groups (one-way ANOVA $F_{(3)} = 2.4$, $p = 0.11$).

The sections were stained with 1% methylene blue, 1% azur B and 1% borax. A Leica DMRA light microscope mounted with a Leica DC300F digital camera and a 63× oil immersion objective was used to take approximately 65 separate micrographs, each of a different part of the auditory nerve. These micrographs were subsequently reassembled and the resulting image was rotated in such a way that

Download English Version:

https://daneshyari.com/en/article/5739400

Download Persian Version:

https://daneshyari.com/article/5739400

Daneshyari.com