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Research paper

Spiral ganglion cell morphology in guinea pigs after deafening and neurotrophic treatment

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ABSTRACT

It is well known that spiral ganglion cells (SGCs) degenerate in hair-cell-depleted cochleas and that treatment with exogenous neurotrophins can prevent this degeneration. Several studies reported that, in addition, SGC size decreases after deafening and increases after neurotrophic treatment. The dynamics of these cell size changes are not well known. In a first experiment we measured size, shape (circularity) and intracellular density of SGCs in guinea pigs at various moments after deafening (1, 2, 4, 6, and 8 weeks) and at various cochlear locations. In a second experiment, the effect of treatment with brain-derived neurotrophic factor (BDNF) on SGC morphology was investigated at various cochlear locations in deafened guinea pigs. We found that SGC size gradually decreased after deafening in the basal and middle cochlear turns. Already after one week a decrease in size was observed, which was well before the number of SGCs started to decrease. After BDNF treatment SGCs became noticeably larger than normal throughout the cochlea, including the middle and apical turns, whereas an effect on survival of SGCs was primarily observed in the basal turn. Thus, both after deafening and after neurotrophic treatment a change in size occurs before survival is affected. Morphological changes were not restricted to a subpopulation of SGCs. We argue that although changes in cell size and changes in survival might be manifestations of two separate mechanisms, morphological measures such as size, circularity and intracellular density are indicative for survival and degeneration.

1. Introduction

Degeneration and protection of the auditory nerve have been studied extensively over the past decades. An important impetus for these studies is the cochlear implant, which relies on a viable nerve in order to be effective in providing electrical hearing in patients with profound sensorineural hearing loss. It is well known that spiral ganglion cells (SGCs) degenerate after loss of cochlear hair cells (Ylikoski et al., 1974; Spoendlin, 1975; Webster and Webster, 1981; Koitchev et al., 1982; Leake and Hradek, 1988; McFadden et al., 2004; Shepherd et al., 2004; Versnel et al., 2007). There is a gradual loss of SGCs, and the cells that survive are smaller and less ovoid-shaped than SGCs in normal cochleas (Staecker et al., 1996; Leake et al., 1999; Richardson et al., 2005; Shepherd et al., 2005; Glueckert et al., 2008; Agterberg et al., 2008). This degeneration of SGCs is thought to be a consequence of loss of neurotrophic support by the hair cells, as the absence of endogenous neurotrophins, such as brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3), leads to a loss of SGCs (Ernfors et al., 1995; Fritzsch et al., 1999; Schimmang et al., 2003). Administration of exogenous neurotrophins can prevent SGC loss (Ernfors et al., 1996; Staecker et al., 1996; Miller et al., 1997; Shinohara et al., 2002; Gillespie et al., 2004; Shepherd et al., 2005; Glueckert et al., 2008; Agterberg et al., 2008; Song et al., 2009; Leake et al., 2011). An additional effect of such treatment is that cells become larger than normal (McGuinness and Shepherd, 2005; Shepherd et al., 2005; Glueckert et al., 2008; Agterberg et al., 2008; Leake et al., 2011).

Abbreviations: BDNF, brain-derived neurotrophic factor; GDNF, glial cell line-derived neurotrophic factor; SGC, spiral ganglion cell; NT-3, neurotrophin-3; $p75^{NTR}$, p75 neurotrophin receptor; TrkB, tropomyosin-related kinase B; ABR, auditory brainstem response; CAP, compound action potential.

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A significant reduction of SGC perikaryal area (by more than 20%) was observed as early as two weeks after deafening, while the number of SGCs remained near-normal (Agterberg et al., 2008; their Fig. 6). Neurotrophic treatment of deafened animals might lead to larger surviving SGCs without enhancement of survival (Richardson et al., 2005). Considering these observations one might say that a change in cell size occurs prior to cell death or cell rescue. In this paper, we address this notion in two experiments in deafened guinea pigs. First, the morphological changes of SGCs after deafening were examined as a function of both time after deafening and cochlear location. Specifically, it was examined whether SGC size or shape changed after one week, when the number of cells is not yet decreased (Versnel et al., 2007). Second, the morphological changes of the SGCs after deafening and subsequent neurotrophic treatment were examined as a function of cochlear location. Dependence on cochlear location is expected for two reasons. First, gradients along the cochlea are found with respect to, among others, expression levels of BDNF and NT-3, and with respect to discharge properties of SGCs (Adamson et al., 2002; Schimmang et al., 2003; Davis and Liu, 2011). Second, delivery of drugs through the round window membrane or through a cochleostomy in the basal turn will allow the drugs to reach the apical turn, albeit in lower concentrations than in the basal turn (Salt and Plontke, 2009; Hahn et al., 2012). Also, the effect of BDNF treatment is often more pronounced in the basal turn (Shepherd et al., 2005; Glueckert et al., 2008; Agterberg et al., 2008); therefore, we examined here whether the effect of BNDF on SGC morphology depends on location in a similar fashion.

2. Materials and methods

2.1. Animals

Thirty-seven albino female guinea pigs (strain: Dunkin Hartley; weighing 250–600 g) were obtained form Harlan Laboratories (Horst, the Netherlands) and housed in the animal care facility of the Rudolf Magnus Institute of Neuroscience. All animals had free access to both food and water and were kept under standard laboratory conditions. Lights were on between 7:00 am and 7:00 pm. Temperature and humidity were kept constant at 21 °C and 60%, respectively. All experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC #03.04.036).

2.2. Experimental design

To describe the morphological changes of SGCs after deafening and the effect of neurotrophic treatment we performed histological analyses of cochleas used in two previous studies of our laboratory (Versnel et al., 2007; Agterberg et al., 2008). Note that in the Versnel et al. study, which described effects of deafening, SGC morphology was not addressed, and in the Agterberg et al. study, which described effects of BDNF treatment, SGC morphology was addressed using electron microscopy but only for one particular cochlear location (upper basal turn).

The treatment schedules of the two experiments are shown in Fig. 1. In the first experiment (Fig. 1A), we quantified the size and shape of SGCs after deafening. Animals were sacrificed at different survival times following the deafening procedure: 1, 2, 4, 6, and 8 weeks. A control group consisting of five guinea pigs was sacrificed at eight weeks after a sham treatment. Except for the 6-week group, all animals were equipped with an electrode consisting of a stainless steel wire with a gold-ball tip positioned in the round window niche of the right cochlea in order to record compound action potentials (CAPs) to tone pips and clicks (for details, see Versnel et al., 2007), which enabled us to measure hearing thresholds. Guinea

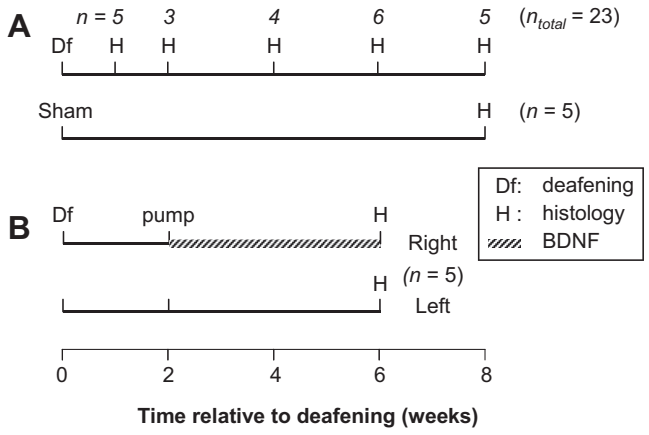

Fig. 1. Treatment schedules of the experiments. (A) Animals were bilaterally deafened and at various times after deafening the animals were sacrificed for histology: 1, 2, 4, 6, and 8 weeks. As control, five animals were sham-treated and examined after eight weeks. In all but the 6-weeks animals, both the right and left cochlea were examined; in the 6-weeks animals only the left cochlea was examined. (B) Five animals were implanted with a mini-osmotic pump and cannula two weeks after deafening. The cannula was inserted in the right cochlea, and BDNF was delivered during four weeks. Immediately thereafter, six weeks after deafening, the animals were sacrificed for histology. Note that the left cochleas were used in the deafening experiment as well.

pigs showing insufficient threshold shifts for click responses (<50 dB), one or two weeks after the deafening procedure, were excluded ($n = 3$). After exclusion, 23 experimental animals remained (for details, see Fig. 1A).

In the second experiment (Fig. 1B) we studied the effect of neurotrophic treatment on the size and shape of SGCs. The right cochleas of six animals were implanted with a cannula two weeks after deafening and were treated with BDNF for four consecutive weeks. The left cochleas remained untreated and were used in the analyses of the first experiment as well. Stainless steel screws were inserted into the skull to record auditory brainstem responses (ABRs) to click stimuli (for details, see Agterberg et al., 2008). Since this study addresses cell morphology given a positive effect of BDNF, one animal was excluded because it did not respond to BNDF treatment (both SGC packing density and SGC size in the treated cochlea were similar to those in the untreated ear; see Fig. 6 in Agterberg et al., 2008). The five remaining animals demonstrated large threshold shifts (>50 dB). They were sacrificed for histology immediately after cessation of BDNF treatment, which was six weeks after deafening (Fig. 1B).

2.3. Deafening procedure

Except for a control group in experiment 1, all animals were deafened. The animals were anaesthetized with ketamine (Ketanest®, 40 mg/kg, i.m.) and xylazine (Sedamun®, 10 mg/kg, i.m.). Kanamycin sulfate in isotonic saline (400 mg/kg) was administered subcutaneously, and the external jugular vein was exposed and cannulated. Furosemide (100 mg/kg, Centrafarm, the Netherlands) was slowly infused, 15–60 min after kanamycin injection. The dose of kanamycin is similar as used in numerous other studies applying kanamycin in combination with ethacrynic acid or furosemide for deafening of guinea pigs (e.g., Staecker et al., 1996; Miller et al., 1997; Nourski et al., 2004; Gillespie et al., 2004; Shepherd et al., 2005; Wise et al., 2005). This procedure, introduced by West et al. (1973), has been shown to eliminate almost all cochlear hair cells when furosemide is infused within 4 h after kanamycin injection (Brummett et al., 1979). The control animals in experiment 1 received isotonic saline (subcutaneously and intravenously), instead of kanamycin and furosemide.