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Glial cell line-derived neurotrophic factor and antioxidants preserve the electrical responsiveness of the spiral ganglion neurons after experimentally induced deafness

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Cochlear implant surgery is currently the therapy of choice for profoundly deaf patients. However, the functionality of cochlear implants depends on the integrity of the auditory spiral ganglion neurons. This study assesses the combined efficacy of two classes of agents found effective in preventing degeneration of the auditory nerve following deafness, neurotrophic factors, and antioxidants. Guinea pigs were deafened and treated for 4 weeks with either local administration of GDNF or a combination of GDNF and systemic injections of the antioxidants ascorbic acid and Trolox. The density of surviving spiral ganglion cells was significantly enhanced and the thresholds for eliciting an electrically evoked brain stem response were significantly reduced in GDNF treated animals compared to deafened-untreated. The addition of antioxidants significantly enhanced the evoked responsiveness over that observed with GDNF alone. The results suggest multiple sites of intervention in the rescue of these cells from deafferentation-induced cell death.

Keywords: Antioxidants; GDNF; Spiral ganglion; Cochlea; Inner ear; Neurotrophic factor; Intervention; Deafferentation; Electrical; Auditory brainstem response

Introduction

Cochlear implants have become widely established as the treatment of choice for profoundly deaf patients who derive little or no benefit from conventional hearing aids. The principle of the cochlear implant is to by-pass the damaged sensory cells and to directly stimulate neural pathways, thus eliciting a sensation of hearing despite missing sensory receptors. The efficacy of cochlear implants is thought to be related to the number and functional state of the remaining spiral ganglion neurons (e.g., Nadol et al., 1989; Incesulu and Nadol, 1998). Thus, interventions to prevent degeneration of auditory sensory neurons would be of therapeutic significance and lead to increased benefits of cochlear implants.

The concept that enhanced survival of spiral ganglion cells provides a better substrate for cochlear implants has been tested in various animal models. The neurotrophic factor hypothesis (see Mattson, 1998 for a review) suggests that deafferentation (in this case, loss of sensory cells) induces a neurotrophic factor deprivation in the afferent nerve fibers, leading to formation of free radicals and up-regulation of cell death pathways. Consistent with that hypothesis, we have demonstrated that a combination of the neurotrophic factors BDNF (brain-derived neurotrophic factor) and CNTF (ciliary neurotrophic factor) applied directly to the inner ear fluids significantly increase both the population of surviving spiral ganglion neurons following deafening, and the efficacy of electrical stimulation as measured by electrically evoked brainstem responses (Shinohara et al., 2002). This is true also if neurotrophic intervention is initiated after delay periods of up to 6 weeks (Yamagata et al., 2004). Ylikoski and colleagues (1998) have previously shown that intracochlear infusion of another neurotrophic factor, GDNF (glial cell-derived neurotrophic factor), enhances the survival of cochlear neurons after noise-induced inner hair cell lesions. They did, however, not investigate the electrical responsiveness of the afferent system. Also the antioxidants ascorbic acid (vitamin C) and Trolox (a water soluble vitamin E analog) reduce deafferentation-induced spiral ganglion cell death (Maruyama et al., 2007), presumably by scavenging neurotrophic factor deprivation-induced free radicals. In these studies, an enhanced survival of spiral ganglion neurons was accompanied by significantly lower thresholds for electrical stimulation.

The purpose of this study was twofold: (1) to test whether GDNF, like BDNF and CNTF, could enhance the electrical

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responsiveness of the spiral ganglion neurons, and (2) to investigate whether a combination of local treatment with GDNF and systemically administered antioxidants would offer additive protection against auditory nerve degeneration in the deafened ear. Our results demonstrate an enhanced electrically evoked responsiveness in the deafened auditory system treated with GDNF, and an even better effect using the combination of GDNF and antioxidants. These findings are significant both for our understanding of the mechanisms of rescue of the auditory nerve from cell death and for the development of interventions to tissue engineer the auditory nerve to enhance benefits of cochlear implants for the severely hearing impaired.

Materials and methods

Experimental design

An overview of the experimental design is shown in Fig. 1. Thirty-two guinea pigs were divided into four groups as follows: (1) Control group; (2) Untreated group; (3) GDNF group; and (4) GDNF + AO group. The Untreated group and the two treatment groups, GDNF and GDNF + AO, were deafened by intracochlear infusion of 10% neomycin for 2 days. This was achieved by using osmotic pumps (Alzet model 2002, Alza Corp., USA) and priming the cannula (connecting to the inner ear) with 24 µl of 10% neomycin (delivered at 0.5 µl/h). For undeafened control animals, the cannula was primed with 24 µl of artificial perilymph (AP) consisting of 0.1% guinea pig serum albumin in lactated Ringer's solution. The 2-day treatment (neomycin or AP) was followed by 4 weeks of delivery of AP for the deafened-untreated group, or GDNF (10 µg/ml in AP; Amgen Inc. USA) or GDNF + AO for the two deafened-treatment groups. In the undeafened-control group and untreated group, AP was administered for 4 weeks via the osmotic pump. The osmotic pump was changed after 2 weeks, and the infusion continued through experimental week 4. The GDNF + AO group received daily intraperitoneal injections of the antioxidants (AO) ascorbic acid (20 mg/kg/day) and Trolox (1 mg/kg/day) for 4 weeks; while all other animals received an equal volume of saline. Trolox (Fluka Chemie, Switzerland) was dissolved in 0.154 N NaOH, neutralized by 0.154 N HCl. The antioxidant solution, prepared immediately before administration, was adjusted to pH 7.2–7.4 with 1 N NaOH after addition of ascorbic acid (Sigma-Aldrich Co. Ltd., Germany).

Subjects

A total of 32 pigmented guinea pigs (250–400 g) with normal Preyer reflexes were used in this study. The animals were anesthetized with xylazine (10 mg/kg i.m.) and ketamine (40 mg/kg i.m.) during the implant surgery and all recording sessions. All animal procedures were performed in accordance with ethical standards of Karolinska Institutet and consistent with national regulations for the care and use of animals (approval no. N113/01).

Implant surgery

All animals underwent aseptic surgery to implant intracochlear stimulating electrodes and an epidural recording electrode as described previously (Maruyama et al., 2007). The left middle ear was exposed by a postauricular approach and a cochleostomy was performed on the basal turn of the cochlea to allow access to scala tympani. A ball electrode was inserted through the round window membrane and placed into scala tympani to elicit electrical auditory brain stem responses (eABRs). The ball electrode was constructed using 75 µm diameter Pt-Ir 90%/10% wire, Teflon-insulated (Advent Research Materials Ltd., England). The return electrode was a Teflon-insulated Pt-Ir wire, 125 µm in diameter (Advent Research Materials Ltd., England). The return electrode was stripped of insulation for 5 mm and placed in the left middle ear. Prior to implantation, each stimulating electrode surface was

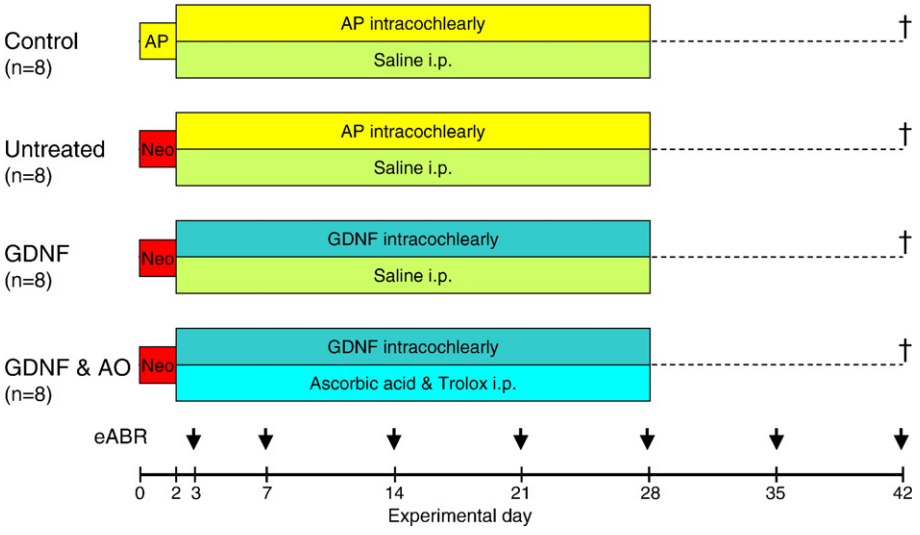

Fig. 1. Graphical presentation of the experimental design. The four groups received identical treatment but were administered different compounds. Immediately after a 2-day infusion into the cochlea (artificial perilymph, AP, or neomysin, Neo), the animals received intracochlear infusions of AP or GDNF for almost four weeks (day 2–28). During the same time period, saline or a mixture of the antioxidants ascorbic acid and Trolox was administered intraperitoneally (i.p.) daily. After a 2-week follow-up period, the animals were sacrificed (†) for histological evaluation. Throughout the experimental protocol, electrically evoked auditory brainstem responses (eABR) were obtained (⬇).