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Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss ☆

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ABSTRACT

Exogenous neurotrophins (NTs) have been shown to rescue spiral ganglion neurons (SGNs) from degeneration following a sensorineural hearing loss (SNHL). Furthermore, chronic electrical stimulation (ES) has been shown to retard SGN degeneration in some studies but not others. Since there is evidence of even greater SGN rescue when NT administration is combined with ES, we examined whether chronic ES can maintain SGN survival long after cessation of NT delivery. Young adult guinea pigs were profoundly deafened using ototoxic drugs; five days later they were unilaterally implanted with an electrode array and drug delivery system. Brain derived neurotrophic factor (BDNF) was continuously delivered to the scala tympani over a four week period while the animal simultaneously received ES via bipolar electrodes in the basal turn (i.e., turn 1) scala tympani. One cohort ($n = 5$) received ES for six weeks (i.e., including a two week period after the cessation of BDNF delivery; ES_6); a second cohort ($n = 5$) received ES for 10 weeks (i.e., a six week period following cessation of BDNF delivery; ES_{10}). The cochleae were harvested for histology and SGN density determined for each cochlear turn for comparison with normal hearing controls ($n = 4$). The withdrawal of BDNF resulted in a rapid loss of SGNs in turns 2–4 of the deafened/BDNF-treated cochleae; this was significant as early as two weeks following removal of the NT when compared with normal controls ($p < 0.05$). Importantly, there was not a significant reduction in SGNs in turn 1 (i.e., adjacent to the electrode array) two and six weeks after NT removal, as compared with normal controls. This result suggests that chronic ES can prevent the rapid loss of SGNs that occurs after the withdrawal of exogenous NTs. Implications for the clinical delivery of NTs are discussed.

1. Introduction

Deafness is one of the most common disabilities in society. In the majority of cases this occurs as a result of loss of sensory hair cells leading to a permanent sensorineural hearing loss (SNHL). Loss of hair cells sets in place a gradual but ongoing degeneration of spiral ganglion cells (SGNs), the primary afferent neurons of the cochlea (Gillespie et al., 2004; Hardie and Shepherd, 1999; Heid et al., 1998; Leake and Hradek, 1988; Liberman and Kiang, 1978; Mair, 1973; McGuinness and Shepherd, 2005; Ryugo et al., 1998; Shepherd and Javel, 1997; Shepherd et al., 2004, 2005; Steel and Bock, 1984; Takeno et al., 1998). SGNs are the target neurons for cochlear implants; the functional integrity of these neurons is therefore important for the success of these neural prostheses.

The removal of afferent input to SGNs removes both the neural activity and neurotrophin support that is normally supplied by hair cells, leading to cell death via apoptosis (Alam et al., 2007; Ladrech et al., 2004; Scarpidis et al., 2003). It is therefore not surprising that attempts to rescue SGNs centre on the use of electrical stimulation (ES) to induce neural activity in these neurons and/or the application of exogenous neurotrophins (NT).

In vitro studies have demonstrated that prolonged periods of depolarization of deafferented SGN cultures via elevated levels of extracellular potassium promotes neural survival via the activation of L-type voltage gated calcium channels (Hegarty et al., 1997; Miller et al., 2003). The resultant elevated intracellular calcium levels activate a number of down-stream pro-survival signalling pathways including cyclic AMP protein kinase, and calcium/

Abbreviations: ABR, auditory brainstem response; ANOVA, analysis of variance; BDNT, brain derived neurotrophin factor; dB, decibel; EABR, electrically-evoked auditory brainstem response; ES, electrical stimulation; ID, internal diameter; IM, intramuscular; IV, intravenous; NT, neurotrophin; NT-3, neurotrophin 3; Pa, Pascal; Pt, platinum; SC, subcutaneous; SEM, standard error of mean; SGN, spiral ganglion neuron; SNHL, sensorineural hearing loss; SPL, standard pressure level; T1, cochlear turn 1 (base); T2, cochlear turn 2; T3, cochlear turn 3; T4, cochlear turn 4 (apex)

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calmodulin-dependent kinases II and IV (Bok et al., 2003; Hansen et al., 2001a; Roehm and Hansen, 2005).

The pro-survival effects of chronic ES of SGNs remain less clear *in vivo*. Using a variety of animal models, a number of studies have demonstrated rescue effects associated with ES (Hartshorn et al., 1991; Kanzaki et al., 2002; Leake et al., 1991, 1992, 1995, 1999; Lousteau, 1987; Miller et al., 1995; Mitchell et al., 1997). In contrast, other studies report no trophic effects associated with ES *per se* (Araki et al., 1998; Coco et al., 2006; Li et al., 1999; Shepherd et al., 1994, 2005; Widijaja et al., 2006); these findings are consistent with a recent temporal bone study of 11 cochlear implant patients that showed no evidence of enhanced SGN survival in the implanted ear compared to the contralateral deafened control ear (Khan et al., 2005).

A number of growth factor families have been shown to play important roles in both the development and maintenance of SGNs (Fritzsch et al., 1999; Rubel and Fritzsch, 2002). Both pre-synaptic hair cells and support cells within the organ of Corti, and post-synaptic neurons within the cochlear nucleus are necessary for SGN survival, reflecting complementary neurotrophic support from both sources (Hafidi, 1999; Lefebvre et al., 1992b, 1994; Schecterson and Bothwell, 1994; Stankovic et al., 2004; Tan and Shepherd, 2006; Tierney et al., 2001; Ylikoski et al., 1993). These NTs include both brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3; Lefebvre et al., 1992a; Schecterson and Bothwell, 1994; Tan and Shepherd, 2006; Ylikoski et al., 1993), with receptors for both of these NTs expressed on SGNs (Schecterson and Bothwell, 1994; Tan and Shepherd, 2006; Ylikoski et al., 1993).

The long-term delivery of exogenous NTs into the cochlea promotes a significant increase in SGN survival following SNHL. Unlike the *in vivo* ES studies described above, the trophic effects of NT delivery on SGNs have been highly significant and universally observed across studies. The majority of these studies delivered BDNF and/or NT-3 to deafened guinea pig cochleae over treatment periods of 2–8 weeks via an osmotic pump (Ernfors et al., 1996; Gillespie et al., 2004; Miller et al., 1997; Richardson et al., 2005; Staecker et al., 1996; Wise et al., 2005; Yamagata et al., 2004), although NT released from alginate beads placed on the round window have also been effective (Noushi et al., 2005). In addition, nerve growth factor (Gillespie et al., 2004; Schindler et al., 1995; Shah et al., 1995), glial cell line-derived neurotrophic factor (Kanzaki et al., 2002; Yagi et al., 2000; Ylikoski et al., 1998) and neurotrophin-4/5 (Gillespie et al., 2004) delivered alone; or ciliary-derived neurotrophic factor (Miller et al., 1997) or fibroblast growth factor (Miller et al., 2007) delivered in combination with other NTs, have also demonstrated SGN rescue *in vivo*. These robust findings have also been reported following NT delivery using viral vectors (Kanzaki et al., 2002; Staecker et al., 1998; Rejali et al., 2007), and in species other than the guinea pig (McGuinness and Shepherd, 2005; Staecker et al., 1998). Pettingill et al., 2007 provides a detailed overview of these studies. Finally, in contrast to long-term NT delivery, a single infusion of NT directly into the scala tympani does not appear to promote long-term SGN survival (Richardson et al., 2005).

Significantly, *in vitro* studies have demonstrated that the trophic support of SGNs via depolarization appears to be additive with the actions of some NTs (Hansen et al., 2001a). This research has been extended to *in vivo* studies, demonstrating enhanced SGN survival in deafened cochleae treated with both exogenous NTs and ES (Kanzaki et al., 2002; Shepherd et al., 2005). Moreover, this work has also shown a functional advantage, in the form of significantly reduced electrically evoked auditory brainstem response (EABR) thresholds in ears treated with NTs (Shepherd et al., 2005; Shinohara et al., 2002), that may be associated with a NT mediated growth of SGN peripheral processes towards the scala tympani (Miller et al., 2007; Staecker et al., 1996; Wise et al., 2005) or reflect the increased diameter of NT treated SGNs (McGuinness and Shepherd, 2005; Shepherd et al., 2005), as threshold to ES decreases with increasing neuron size (Grill, 2004).

The duration of the exogenous NT supply is a key issue that impacts on the safe clinical delivery of these drugs. Although there is evidence that SGN survival is maintained well after cessation of NT administration (Miller et al., 2006), other research has demonstrated that the withdrawal of NT support leads to an *accelerated* loss of SGNs (Gillespie et al., 2003) – a finding that implies that NTs must be supplied continuously. Given the positive results of combining ES and NT delivery, we have examined whether chronic intracochlear ES can provide trophic support to SGNs long after removal of the source of exogenous NTs.

2. Material and methods

2.1. Experimental subjects

Twelve healthy young pigmented guinea pigs weighing between 400 and 844 g (mean 569 ± 133 g) were used in the present study under approval of the Royal Victorian Eye and Ear Hospital's Animal Research and Ethics Committee, and conformed to the guidelines of the National Health and Medical Research Council of Australia.

All animals had otoscopically normal tympanic membranes and normal hearing determined by click-evoked auditory brainstem response (ABR) thresholds of <43 dB peak equivalent sound pressure level re 20 μPa (p.e. SPL; Hardie and Shepherd, 1999). Guinea pigs were systemically deafened using a single co-administration of kanamycin (400 mg/kg subcutaneously [sc]) and frusemide (100 mg/kg intravenously [iv]; Gillespie et al., 2003). Only animals exhibiting a severe-profound SNHL (i.e., ABR click thresholds >93 dB p.e. SPL in both ears) were used in this study. The animals were divided into two treatment groups. Both groups received BDNF over a four week period in combination with chronic ES delivered for either six (ES_6; $n = 5$) or 10 weeks (ES_{10}; $n = 5$; Table 1). The remaining animals served as normal hearing controls ($n = 4$ cochleae).

2.2. Electrode array and delivery techniques

The electrode array consisted of three platinum (Pt) band electrodes on a 0.6 mm diameter silicone carrier. Each Pt electrode was connected to a stainless-steel leadwire system via a 25 μm diameter platinum/iridium (90/10) wire (Shepherd and Xu, 2002). A Pt marker was located 5 mm from the tip of the array as a guide to insertion depth. A 0.75 mm internal diameter (ID) polyvinyl chloride tube connected a mini-osmotic pump (Alzet 2004) to a 0.124 mm ID delivery-tube located within the central core of the electrode array. The contents of the osmotic pump were delivered to the scala tympani of the cochlea through a lumen in the delivery-tube at the tip of the array (Shepherd and Xu, 2002).

2.3. Preparation of the osmotic pump

The Alzet 2004 mini-osmotic pump has a reservoir capacity of 200 μl and a flow rate of 0.25 μl/h, providing a continuous

Table 1
Summary of treatment groups

Treatment group	Total implant duration (weeks)	Duration of BDNF treatment (weeks)	Duration of ES alone (weeks)
ES_6	6	4	2
ES_{10}	10	4	6