Function and expression pattern of brain derived nerve growth factor in spiral ganglion: Implication for research on cochlea implants

Abstract

Background; As previously various studies have suggested application of brain brain-derived neurotrophic factor (BDNF) may be considered as a promising future therapy for hearing deficits, in particular for the improvement of cochlear neuron loss during cochlear implantation (1,2).

Aims; The present study aimed to find out the threshold of brain-derived neurotrophic factor

(BDNF) in spiral ganglion outgrowth. Our suggestion for hydrogel concentration should be 25ng/ml.

Study design; Original article (experimental animal trials).

Methods: Spiral ganglion explants were prepared from postnatal day 4 (p4) NMRI mice (outbred model was developed by Lynch et al. in 1937. The mice were inbred as NIH/P1) of both sexes under the approval and guidelines of the regional council (Hearing research institute Tubingen). The skin was removed and the skull was opened midsagitally. After removal of the brain the temporal bone was harvested and transferred to a sterile petri dish filled with ice cooled dissection buffer hepes-hanks solution. The bony capsule of the cochlea was removed exposing the membranous parts of the cochlea. The Stria vascularis, the organ of Corti and the bone around the modiolus were removed separating the entire spiral ganglion. (3). For brain-derived neurotrophic factor (BDNF) detection on spiral ganglion explants we used primary antibody: neurofilament 200 (NF200), glial fibrillary acidic protein (GFAP). As secondary antibody we used cyanine (Cy3) and Alexa 488.

Results; Spiral ganglion explants were cultured at postnatal days 4 in the presence of different concentrations of BDNF as described under methods. We choose an age of post natal day (P4) and concentrations of brain-derived neurotrophic factor (BDNF) 0; 6, 12.5; 25 and 50 ng /ml. From 4 different cultures an averaged neurite could be measured. What can be shown is that with increasing concentrations of brain-derived neurotrophic factor (BDNF) the neurite density and length apparently increases.

To test the endogenous expression of BDNF we used crosections of a mice model BDNF LIVE EXON VIEWING (BLEV) that was previously generated to visualize the activity dependent usage of different promoters upstream the BDNF Exon IV (CFP) and Exon VI (YFP) of the BDNF gene (Singer et al., Knipper, in preparation). When the immature cochlea of BLEV heterozygous mice at P6 were prepared as described for the culture cochlea explants in the previous chapter, a clear expression of both YFP BDNF exon VI (Fig. 22A, 22C) and CFP BDNF exon IV (Fig. 26B, 26D) could be shown in Spiral Ganglia neurons that were costained with Propidium Bromid (Fig. 26B, 26D). Also as YFP (Fig. 22A, arrow) and CFP (Fig. 22C, arrow) fluorescence indicate BDNF targeting to dendrites.