USING HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) MEDIATED GENE TRANSFER TO STUDY NEUROTROPHINS IN COCHLEAR NEURONS

Juan Jose GARRIDO, Maria Teresa ALONSO, Filip LIM, Juan REPRESA, Fernando GIRALDEZ and Thomas SCHIMMANG

Instituto de Biología y Genética Molecular (IBGM)
Universidad de Valladolid-CSIC, Valladolid, Spain

The aim of this work was to gain further insight in the potential therapeutic role for neurotrophic factors in cochlear ganglion neurons. It is well known that cochlear neurons are dependent on members of the nerve growth factor family called neurotrophins for their survival (Avila et al., 1993) and maintenance of target innervation (Schimmang et al., 1995). The cochlear sensory epithelia composed of inner and outer hair cells are the source of two of these neurotrophins, namely brain-derived neurotrophic factor (BDNF) and neurotrophin-3. The recent analysis of knockout mice for these factors and their high-affinity receptors termed Trks have proven their essential function for cochlear development (Ernfors et al., 1995; Minichiello et al., 1995).

Defective Herpes Simplex Virus Type 1 (HSV-1) plasmid vectors, or amplicons, permit the transfer and study of genes into mammalian neurons. In the present study we first demonstrate that HSV-1 vectors can also be used to transfer and express genes in avian neurons. Next, we have infected these neurons with a defective HSV-1 vector carrying BDNF. The data presented here show that gene transfer using this vector leads to neurite outgrowth, reflecting the expression of biologically active BDNF in the infected cells.

Cochlear neurons were prepared as follows: Cochlear ganglia from chicken embryos of stages E31 to E35 stages were isolated, placed in DMEM/F12 and washed in HBSS without Ca^{2+} or Mg^{2+}. Dissociation was carried out using 0.05% trypsin in HBSS without Ca^{2+} or Mg^{2+} for 15-20 min at 37° C. Cells were washed in DMEM/F12 with 10% FCS, dissociated using a fire polished Pasteur pipette and centrifuged at 800 rpm for 2 min in a benchtop centrifuge. The medium was removed and cells were resuspended in medium (DMEM/F12 supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 6 mg/ml glucose, 60 ng/ml progesterone, 16 µg/ml putrescine and 30 nM sodium selenite) and seeded in poly-L-lysine (100 µg/ml) and laminin (10 µg/ml) coated culture dishes at a final density of 1000-2000 cells per well. After two hours at 37°C in a 5% CO_2 atmosphere medium was changed to reduce the serum concentration to 1% and BDNF (5 ng/ml) or pHSVlac or pHSVbdnf virus was added to the medium. Neurons were cultured for 48 hours, fixed in 4% paraformaldehyde in PBS and stained with a neurofilament (160 kDa) or the A2B5 antibody. The percentage of neuritogenesis observed was used as the parameter to measure the effect of the different conditions.

Construction, packaging and titering of the pHSVlac and pHSVbdnf vector have been described previously (Alonso et al., 1996 and references therein). As helpervirus the HSV-1 IE2 deletion mutant 5dl1.2 was used with a titer of 2×10^7 infectious vector units/ml (Lim et al., 1996). Neurons were infected with 2.4×10^5 ivu/ml and 1.4×10^5 ivu/ml of pHSVlac or pHSVbdnf, respectively.

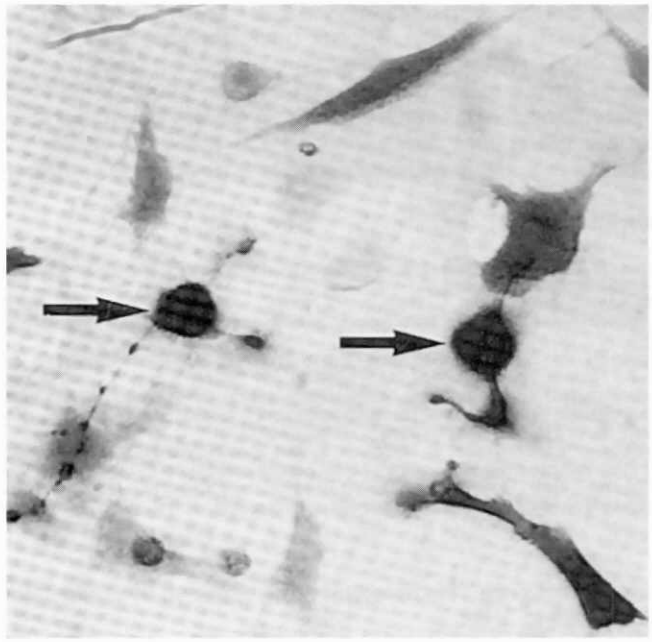

Figure 1. cultures of isolated chicken auditory neurons of stage E35 transduced with pHSVlac. Arrows point at neurons expressing β-galactosidase

To determine the abilty of HSV-1 virus to infect chicken auditory neurons we used the prototype HSV-1 vector pHSV lac containing a E. coli lacZ marker gene. Neurons from various stages were isolated, infected with pHSVlac, and 2 days later an in situ assay for β-galactosidase was performed. High expression of β-galactosidase was detected in almost all neurons, indicating efficient infection of cells (Figure 1).

We next transduced auditory neuron cultures with pHSVlac or pHSVbdnf, which contains a cDNA encoding human BDNF. After 48 hours cultures were fixed and stained with antibodies to analyse the extension of neurites. While cultures infected with pHSVlac only contained few neurons with neurites comparable with untreated control cultures, pHSVbdnf induced neuritogenesis in 60% of the cochlear neurons (Figure 2 and data not shown). The latter effect was comparable with the maximal effects obtained by treating neurons with recombinant BDNF. Transfer of conditioned medium from cultures infected with pHSVbdnf to control cultures was also able to reproduce the neuritogenesis seen in pHSVbdnf infected neurons. BDNF expressed by pHSVbdnf is therefore producing direct and specific effects on auditory sensory neurons.

In summary, we conclude that chicken cochlear neurons can be infected with defective HSV-1 vectors without showing cytopathic effects. Expression of BDNF inside these neurons results in outgrowth of neurites in the majority of cells reflecting the known biological activity of this factor.

Auditory neurons are targets for damage by noise and different ototoxic drugs, resulting in loss of their innervation with the sensory epithelia. Specific neurotrophins, including BDNF have recently been shown to protect cochlear neurons from ototoxic damage (Gao et al., 1996). HSV-1 mediated transfer of neurotrophins may be envisaged as a possible therapeutic tool, allowing the recovery and/or protection of auditory neurons during or after damage.

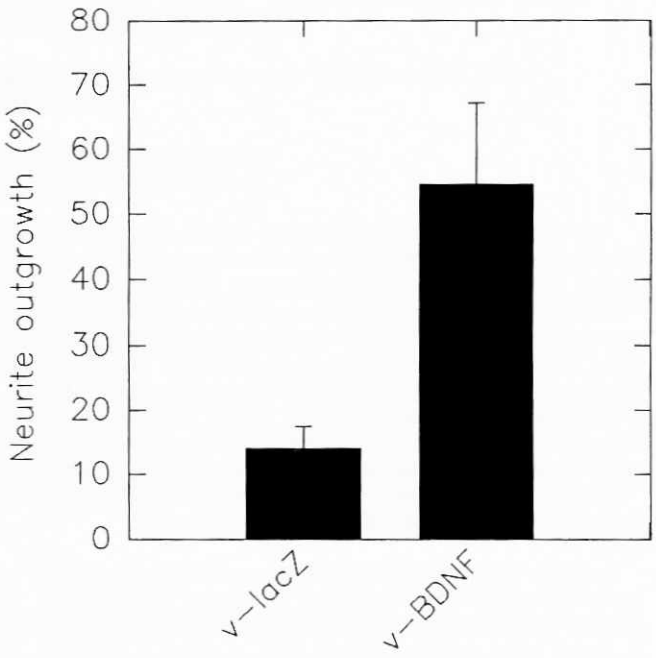

Figure 2. Effects on neurite outgrowth measured after infection with pHSVlac and pHSVbdnf.

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