The interactions between the cytokine LIF and the neurotrophins on spiral ganglion cells

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SUMMARY

The survival of auditory neurones depends on the continued supply of trophic factors. Early post-natal spiral ganglion cells (SGC) in a dissociated cell culture were used as a model of auditory innervation to test the trophic factors leukaemia inhibitory factor (LIF) and neurotrophin-3 (NT-3) for their ability, individually or in combination, to promote neuronal survival.

INTRODUCTION

Degeneration of spiral ganglion cells (SGC) is one of the most common histopathologic correlates of sensorineural hearing loss. Studies show that hair cells within the cochlea produce and supply trophic factors. As a result of trauma, disease or ageing, cochlear hair cells are lost, leading to a secondary wave of SGC degeneration, suggesting that the continued supply of growth factors is responsible for maintaining neuronal integrity.

The neurotrophin brain derived neurotrophic factor (BDNF) and NT-3 have been reported to exert activity on SGC, providing protection against cisplatin toxicity and promoting neuronal survival (Zheng et al. 1995). In addition, BDNF and NT-3 have been reported to play a role in the development and patterning of developing auditory neurones (Furtilas et al. 1994; Stancker et al. 1996).
The cytokine and growth factor ciliary derived neurotrophic factor (CNTF) has also been reported to have trophic effects on SGC (Staecker et al. 1995). In addition, a combination of CNTF and BDNF were reportedly more effective in promoting the survival of rat SGC in cultures than either factor individually (Hartnick et al. 1996). Leukaemia inhibitory factor (LIF) is a cytokine structurally homologous to CNTF. Furthermore, the receptors for LIF and CNTF also share two identical components, which are believed to induce their overlapping biological activity and signal transduction (Pap and Yancopoulos 1996).

Therefore, we wanted to test if LIF could potentiate neuronal survival in the mammalian cochlea. Moreover, we wanted to assess if additive or synergistic effects were occurring with respect to neuronal survival when neurotrophin and LIF were added in combination.

MATERIALS AND METHODS

Cochleae were dissected from five-day-old (P5) rat pups (Wistar). After removal of the organ of Corti the modiolus was digested in HEM, containing 0.025% trypsin (Sigma) and 0.001% DNase (Sigma) (30 min at 37 °C). The trypsinisation was terminated by the addition of 1 ml fetal bovine serum (FBS). Digested cochleae were centrifuged (1500 × g for 5 min at room temperature (RT)) and the pellet was gently triturated in HEM containing 0.001% DNase. The SGC suspension was again centrifuged (1500 × g for 5 min at RT) and the pellet resuspended in Dulbecco modified Eagle's media (DMEM) + N1 supplement + glucose adjusted to a final concentration of 4.5 g/l.

The dissociated SGC suspension was pre-plated in 35 mm tissue culture plates (Falcon) (30 min at 37 °C, 10 % CO₂). The supernatant of the enriched SGC was collected and a cell count was performed on 10 III of SGC suspension. The SGC suspension was again centrifuged (1500 × g for 5 min at RT) and the pellet resuspended in Dulbecco modified Eagle's media (DMEM) + N1 supplement + glucose adjusted to a final concentration of 4.5 g/l (glucose). Growth factors were added to the media at time of plating at a range of concentrations. To determine the initial seeded number of SGC/well a separate plate was incubated at 37 °C, 10 % CO₂ for 4 hr, the cells were then fixed and stained using the avidin-biotin complex (ABC) method (Vectorstain kit). The cultures were fixed in 100% methanol (30 min) then rinsed in phosphate buffered saline (PBS). Non-specific binding was prevented by the addition of 2% FBS in PBS (RT for 30 min). The cultures were incubated with the primary antibody: anti-200 kDa neurofilament (RT for 30 min). The cells were then reacted with ABC reagents according to manufacturer's protocol, and then stained for 5 min at RT with diaminobenzidine (DAB). Surviving neurones were defined as cells whose somas immunostained with the αNF 200 antibody and possessed neuritic projections that were at least equal in length to three-times the width of the neuronal soma. To measure neuronal survival all the immunostained neurones in each well was counted. The data were analysed for significance using ANOVA on Ranks and Student-Newman-Keuls method for multiple comparison.

RESULTS

Dissociated SGC were plated at 10³ cells/well with a mean seed number of 518 ± 6 neurons per well (mean ± SEM). After 3 days in vitro the average number of viable cells (or cells with projections) in the untreated wells had decreased to 11 ± 2 cells per well, representing a survival rate of 2.1 % compared to the neuronal population after 4 hours of plating (seed). The addition of LIF promoted the survival of SGC in a concentration-dependent manner, with a significant increase in neuronal survival detected at concentrations as low as 0.1 ng/ml compared to the untreated wells (see Fig. 1). Maximum neuronal survival using LIF alone was at 10 ng/ml (54 ± 5 cells/well). At higher concentrations (ie 100 ng/ml), there was a significant decrease in the survival of SGC (p < 0.05; Student-Newman-Keuls pairwise comparison procedure).

When used concurrently with NT-3, LIF retained a concentration-dependent survival promoting efficacy, with a maximal survival promoting effect at a concentration of 10 ng/ml/50 ng/ml (LIF/NT-3). In addition, LIF and NT-3 showed significant survival activity at concentrations as low as 0.1 ng/ml.0.1 ng/ml (LIF/NT-3). When combined, the survival promoting action of LIF and NT-3 appeared to be synergistic (see Fig. 1), since the survival percentage of the combination was 1.6-fold greater than the aggregate of the maximal increases recorded when using each factor alone.

DISCUSSION

This study indicates that LIF individually or in combination with the neurotrophin NT-3 can promote survival of SGC in culture. At present it is not known what role LIF plays in the auditory system. LIF's known pleiotropic activity across other classes of neurones (Hilton et al. 1991), suggests that it may also have various roles within the auditory system, such as in neurogenesis, maintenance of neuronal integrity and/or injury response.

With respect to a role in neuronal development, studies report that LIF mRNA has been detected in the developing dorsal root ganglia (DRG) of rat at a time when neuronal differentiation is occurring, and that LIF and NGF interact additively in the development of DRG neurones cultures (Murphy et al. 1993). In addition, studies
have detected the distribution of \[^{125}I\]LIF binding sites in both neural crest- and placode-derived sensory ganglia (Qui et al. 1994). Because SGC derive from the otic placode, while Schwann cells and supporting cells are from neural-crest origin, it is possible that during embryogenesis these cells could also express \[^{125}I\]LIF binding sites, and that LIF could have a developmental role in the cochlea. We have detected the distribution of \[^{125}I\]LIF binding sites in both neural crest- and placode-derived sensory ganglia (Qui et al. 1994). Because SGC derive from the otic placode, while Schwann cells and supporting cells are from neural-crest origin, it is possible that during embryogenesis these cells could also express \[^{125}I\]LIF binding sites, and that LIF could have a developmental role in the cochlea.

At present it is unknown if LIF may also have a target-derived neurotrophic role (expressed in hair cells). It has been reported that the expression of LIF mRNA in rat brain and peripheral tissue was selectively localised in target areas of developing sympathetic neurones (Yamamori et al. 1991). It was proposed that one of LIF's pleiotropic actions may be as a target-derived factor (Yamamori et al. 1991). This hypothesis is corroborated by LIF's retrograde transport from the target tissue (Kurek et al. 1996). Recent studies show that seven families of growth factors and their respective receptors are found in the post-natal cochlea, including the CNTF family of factors (Malgrange et al. 1997). We wait further in situ studies to determine if LIF is also expressed by this system, and thus ascertain if LIF may also have a target-derived neurotrophic role within the adult cochlea.

It is unknown if the potentiating effect of LIF upon NT-3-induced survival is occurring on the same neurones. The converging second messenger pathways for the neural cytokine and neurotrophin, and the synergistic action demonstrated in these studies suggest that LIF and NT-3 may indeed be acting on the same SGC to potentiate each other's survival effects. Conversely, in dissociated cell cultures other non-neuronal cells (i.e. fibroblast and glial cells) proliferate, and therefore, it is possible that LIF may act via these cells as well as exert direct action on the SGC themselves. In addition, studies with antibodies directed at the trkB and trkC receptors for the neurotrophins revealed that in vitro there are populations of SGC that are neurotrophin-independent (Zheng et al. 1995). Thus the population of surviving neurones may include cells targeted by both factors, and cells supported by only one of the factors. Double labelling studies may elucidate the site(s) of LIF-receptor and thus the site(s) of action.

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Title:
The interactions between the cytokine LIF and the neurotrophins on spiral ganglion cells

Date:
1997

Citation:

Persistent Link:
http://hdl.handle.net/11343/26998

File Description:
The interactions between the cytokine LIF and the neurotrophins on spiral ganglion cells

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