Introduction: Expression of the pro-inflammatory cytokine interleukin-1 beta (IL-1β) is increased following nervous system injury. Generally IL-1β induces inflammation, leading to neural degeneration, while several neuroprotective effects of IL-1β have also been reported, for example, promotion of Schwann cell proliferation [1], increase of expression of nerve growth factor [2], and promotion of neuronal survival [3]. Although neurite outgrowth is an important step in nerve regeneration, whether IL-1β takes advantages on it is unclear. We examine how IL-1β is expressed following sciatic nerve injury in rats, and how it affects on neurite outgrowth.

Materials and Methods: Dorsal root ganglion (DRG) neurons from P9 or 10 Wistar rats and cerebellar granule neurons (CGNs) were cultured on poly-L-lysine coated culture dishes or 4-well chambers in Sato medium. For neurite outgrowth assay, DRG neurons were cultured for 72 hours with IL-1β (50 ng/ml), myelin associated glycoprotein (MAG; 25 mg/ml) or p38 MAPK inhibitor SB203580 (1 mM), and performed immunofluorescence using anti-TUJ1 antibody. The longest neurite of TUJ1-positive neuron was measured using an image analyzer. For Rho assay, CGNs were lysed in lysis buffer, and the cell lysates were incubated for 45 min with Rho Assay Reagent (Upstate) to selectively bind activated RhoA for the pull-down assay. Whole-cell lysates were directly immunoblotted to determine the total amount of RhoA. RhoA was detected by Western blotting using anti-RhoA antibody.

Results: Neurite outgrowth was estimated using immunofluorescence.

Figure 1

![Figure 1](image)

Figure 1 Effects of IL-1β on neurite outgrowth in DRG neurons. (A) Axonal lengths were measured using MAG or IL-1β. (B) Axonal lengths were measured using DMSO, SB203580, or IL-1β. Error bars indicate the mean ± SE. * p<0.05 and p<0.01. Results are representative of 3 independent experiments.

MAG inhibited neurite outgrowth (138.6 ± 7.8 μm) compared with the control (176.3 ± 7.1 μm), while IL-1β with MAG enhanced it (180.4 ± 11.0 μm) compared with MAG (Fig. 1 A). Addition of IL-1β alone did not change axonal length (176.5 ± 7.5 μm). These findings suggest that IL-1β promoted neurite outgrowth by inhibiting the effects of MAG.

In neurons, Neurite outgrowth is regulated by the small GTPase RhoA, activation of which leads to the inhibition of neurite outgrowth. We therefore examined RhoA activity by Rho pull-down assay using CGNs (Fig. 2 A).

Discussion: Observations in the present study reveal that IL-1β promotes neurite outgrowth by inhibiting Rho activity, and that p38 MAPK mediated IL-1β-induced neurite extension. Thus, IL-1β may contribute to nerve regeneration by promoting neurite outgrowth after nerve injury.

Several effects of IL-1β on non-neuronal cells have been reported. In Schwann cells, IL-1β promotes proliferation [1]. During Wallerian degeneration after peripheral nerve injury, Schwann cells must proliferate linearly, forming bands of Büngner, which support axonal outgrowth. Moreover IL-1β increases the synthesis of NGF [2], which promotes neuronal survival and axonal elongation indirectly as well as in the direct fashion indicated by our findings. IL-1β also induces migration of macrophages and then aids myelin clearance, which facilitates axonal outgrowth [4]. These effects suggest that IL-1β plays an essential role during the early stage of Wallerian degeneration.