The Effects of Neuronal Growth Factors on LPS-Activated Schwann Cells

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Introduction

Schwann cells (SCs) are the principal support cells of neurons in the peripheral nervous system, that both myelinate axons for the rapid conduction of electrical impulses as well as assist in axonal repair during nerve injury. During nerve injury, SCs secrete tumor necrosis factor alpha (TNF-α)[1,5,6] and other proinflammatory mediators[1,6], attracting macrophages to the site of injury to induce inflammation and clear myelin debris.[1,6] Once the debris is cleared, the neuron stimulates SC proliferation by secreting neuronal mitogens, such as heregulin[3,4], and an unknown factor that activates the cAMP pathway[3], an important regulator of cell division.[3,4] *In vitro*, SCs can be treated with an artificial plant extract called forskolin[3,4] to activate the cAMP pathway. Studies show that heregulin and forskolin act synergistically to enhance SC proliferation under normal, non-inflammatory conditions.[4] Although the role of cAMP in proliferation and axonal regeneration is well-known, not much has been explored about its function in SCs during nerve injury and inflammation. *In vitro*, inflammatory conditions can be simulated by treating SCs with lipopolysaccharide (LPS), a cell-wall immunostimulatory component of Gram-negative bacteria.[1,2,6] In most mammalian cells, LPS binds to a transmembrane protein called toll-like receptor 4 (TLR4)[1,2,6], activating both the mitogen-activated protein kinase (MAPK) pathway[1,2,6] and the nuclear factor kappa B (NF-κB) pathway[2,6], to promote the secretion of inflammatory mediators, such as TNF-α.[1,2,6] With that being said, the aim of this preliminary study was to determine the role of the cAMP pathway in SCs during LPS-induced inflammation. It was hypothesized that SCs stimulated with LPS and growth factors will have higher proliferation than SCs treated with LPS only.

Schwann Cell Morphology in Response to LPS

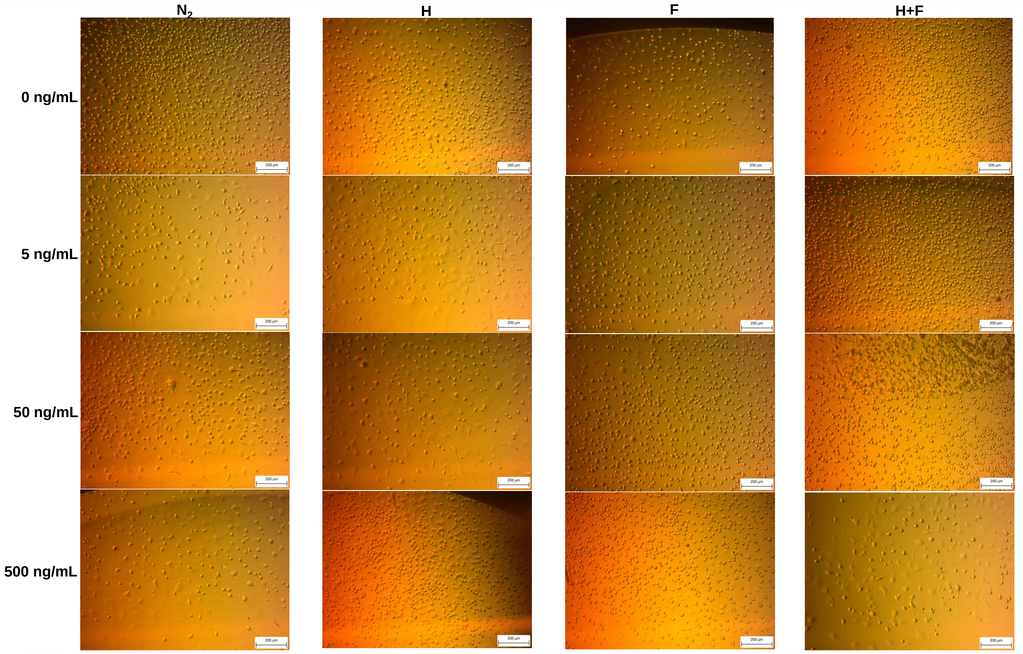

Figure 1. Effects of 24-hour LPS treatment with or without growth factors on SC morphology. S16 SC line (SC-2941) was cultured in control media (N_2), 12.5 ng/mL heregulin (H), 2 mM forskolin (F), or H+F, and 0, 5, 50, or 500 ng/mL LPS. The above images are a representative set of images from a 24-hour incubation with LPS. For the 24-hour incubation, cells treated with H+F and 5 ng/mL LPS exhibited the most proliferation, whereas cells treated with N_2 and 500 ng/mL LPS exhibited the least proliferation. The experiment contained three independent trials for 1, 3, 12, and 24-hour incubations with LPS.

Schwann Cell Proliferation in Response to LPS

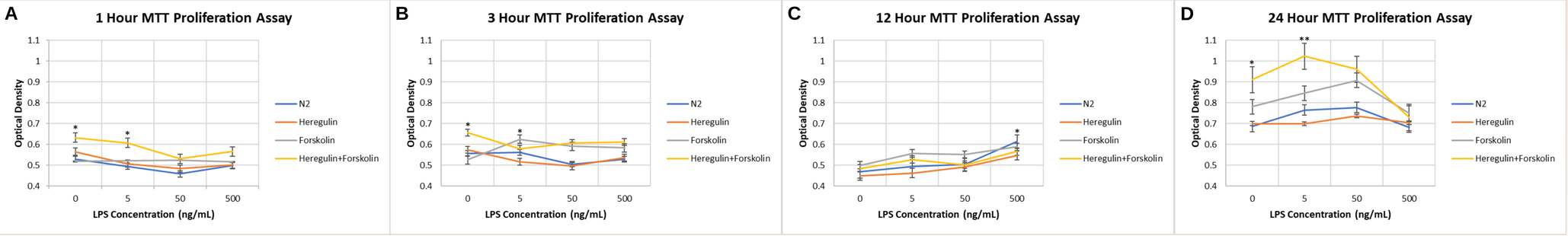

Figure 2. Effects of LPS treatment with or without growth factors on SC proliferation. Using an MTT proliferation assay, the S16 SC line (SC-2941) was treated for 1 (A), 3 (B), 12 (C), or 24 (D) hours, with no growth factors (control media, N_2), 12.5 ng/mL heregulin (H), 2 mM forskolin (F), or H+F, and various doses of LPS at 0, 5, 50, or 500 ng/mL, in 96-well plates. The optical density of each treatment was read at 570 nm as an indicator of cell proliferation and is displayed as mean ± SEM. Results from all four experiments were examined using one-way ANOVA and tested with LSD post-hoc analysis. *P < 0.05 and **P < 0.01, compared to other treatments within the same time point (n = 3).

Conclusion

Between the treatments used, it appears as though 5 ng/mL of LPS combined with both heregulin and forskolin for 24 hours results in the most significant SC proliferation. Considering SCs treated with 5 or 50 ng/mL of LPS for 24 hours had generally higher optical densities than cells treated with 5 or 50 ng/mL of LPS for 1, 3, or 12 hours, there may be some period of recovery between 12 and 24 hours at lower LPS doses. These findings suggest that, when SCs are activated with low doses of LPS for 24 hours, heregulin and forskolin together may act to overcome the decrease in proliferation initiated by the LPS/TLR4 pathway.

References

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Acknowledgements

The authors wish to acknowledge the Misericordia University Summer Undergraduate Research Fellowship for financial support. The authors would also like to acknowledge Kyle Kenney, Leo Carr, Jill Dillon, Helen Bogdon, Daria Fitz, and the rest of the Misericordia University Department of Biology for their assistance with this research.