The Role of Schwann Cells in Nerve Injury

Forskolin-mediated cAMP activation upregulates TNFα expression despite NF-κB downregulation in LPS-treated Schwann cells

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Introduction

What are Schwann cells?

- Glial cells of peripheral nervous system that produce myelin sheath
- Involved in axonal regeneration following nerve injury[4]
- Two potential mechanisms: NF-κB and cAMP pathways

The NF-κB Pathway:

- Involved in innate and adaptive immunity[5]
- Produces tumor necrosis factor (TNFα) and other cytokines[2,3,5]
- Stimulated by lipopolysaccharide (LPS), a bacterial endotoxin[2,3,5]

The cAMP Pathway:

- Involved in cell proliferation[1] and has anti-inflammatory effects[6]
- Interacts with A-kinase-anchoring protein 95 (AKAP95)[1,6] and cyclin D3[1]
- Simulated by forskolin, an artificial plant extract[1]

Research Questions:

1. Do LPS-treated cells with forskolin have more **viability** than cells without forskolin?
2. Do LPS-treated cells with forskolin have less **NF-κB and TNFα expression** than cells without forskolin?
3. Do LPS-treated cells have more **AKAP95 and cyclin D3 expression** than cells without forskolin?

Preliminary Findings

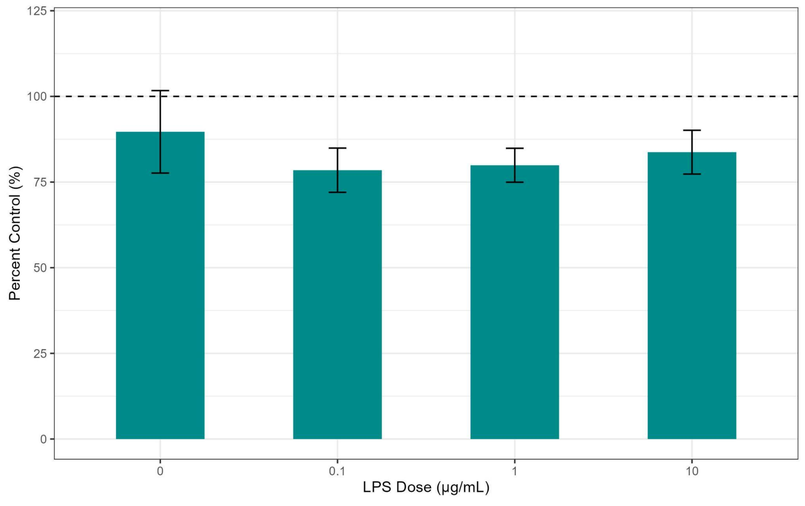

Figure 1. The effects of LPS with or without forskolin on Schwann cell viability. Using the CyQUANT™ MTT Cell Viability Assay Kit (Thermo Fisher), the immortalized rat RT4-D6P2T cell line (ATCC #CRL-2768) was treated with 0, 0.1, 1, or 10 μg/mL of LPS, with or without 2 μM of forskolin, for 3 hours. Optical density was read at 570 nm as an indicator of cell viability and is displayed as mean percent control ± SEM. There is a dotted line to indicate a mean percent control of 100%. Values above the line mean forskolin increased cell viability, while values below the line mean forskolin decreased cell viability. Results from three independent experiments (n = 3) were examined using one-way ANOVA and tested with Tukey's and LSD post-hoc analyses in R Studio. There were no significant differences in mean percent control between the various doses of LPS (F = 0.986, df = 1, p = 0.328).

Results

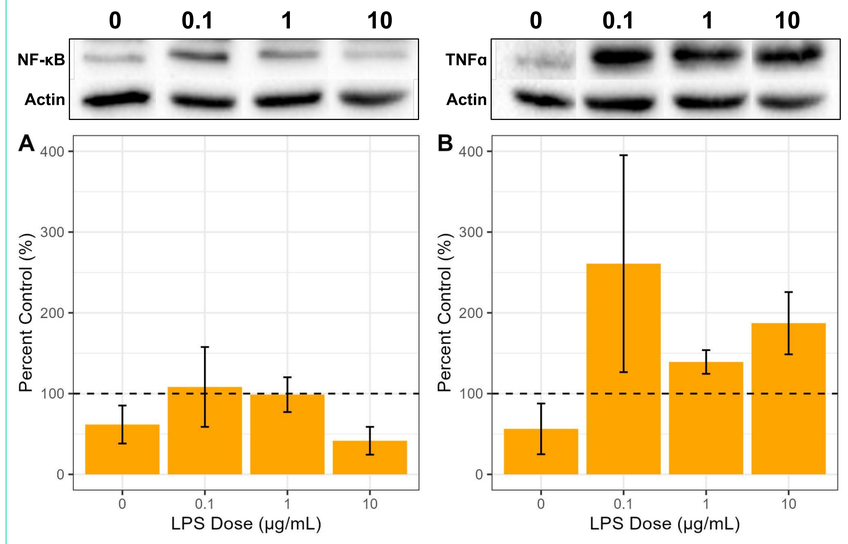

Figure 2. The effects of LPS with or without forskolin on NF-κB (A) and TNFα (B) expression in Schwann cells. The immortalized rat RT4-D6P2T Schwannoma cell line (ATCC #CRL-2768) was treated with 0, 0.1, 1, or 10 μg/mL of LPS, with or without 2 μM of forskolin, for 3 hours. SDS-PAGE gel electrophoresis and Western blot were performed using prepared cell lysates. NF-κB and TNFα expression were visualized using enhanced chemiluminescence reagent and quantified via densitometry analysis using Bio Rad Image software. Actin was used as a loading control. The above blots are representative blots from three independent experiments. Relative band densities are displayed as mean percent control ± SEM. Results from three independent experiments (n = 3) were examined using one-way ANOVA and tested with Tukey's and LSD post-hoc analyses in R Studio. For NF-κB expression, there were no significant differences in mean percent control between the various doses of LPS (F = 1.812, df = 1, p = 0.208). For TNFα expression, there were also no significant differences in mean percent control between the various doses of LPS (F = 0.144, df = 1, p = 0.713).

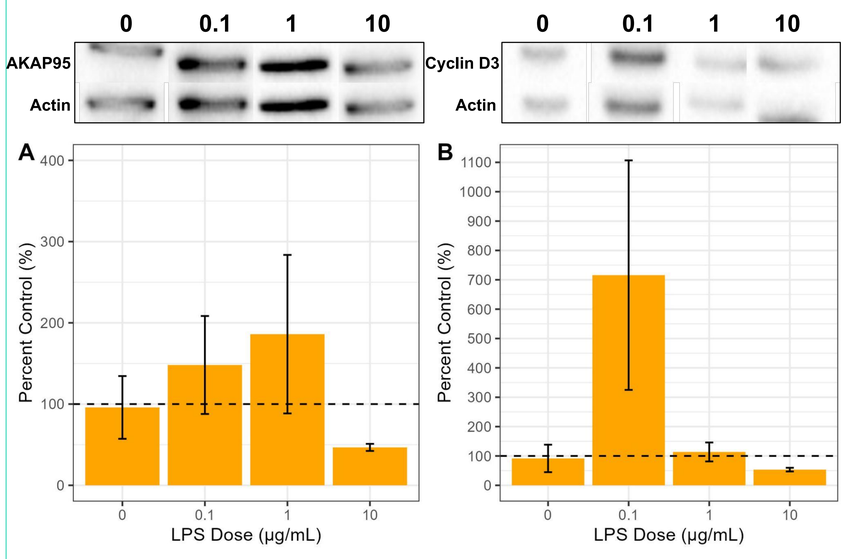

Figure 3. The effects of LPS with or without forskolin on AKAP95 (A) and Cyclin D3 (B) expression in Schwann cells. The immortalized rat RT4-D6P2T Schwannoma cell line (ATCC #CRL-2768) was treated with 0, 0.1, 1, or 10 μg/mL of LPS, with or without 2 μM of forskolin, for 3 hours. SDS-PAGE gel electrophoresis and Western blot were performed using prepared cell lysates. AKAP95 and Cyclin D3 expression were visualized using enhanced chemiluminescence reagent and quantified via densitometry analysis using Bio Rad Image software. Actin was used as a loading control. The above blots are representative blots from three independent experiments. Relative band densities are displayed as mean percent control ± SEM. Results from three independent experiments (n = 3) were examined using one-way ANOVA and tested with Tukey's and LSD post-hoc analyses in R Studio. For AKAP95 expression, there were no significant differences in mean percent control between the various doses of LPS (F = 1.782, df = 1, p = 0.212). For Cyclin D3 expression, there were also no significant differences in mean percent control between the various doses of LPS (F = 1.011, df = 1, p = 0.338).

Conclusions & Future Research

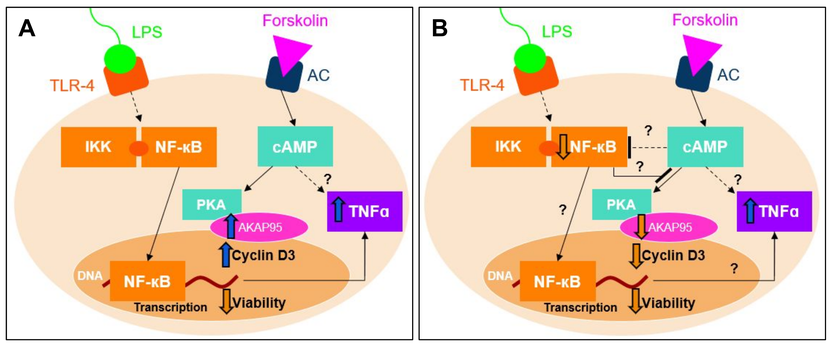

Figure 4. Illustration of the potential mechanisms by which forskolin-mediated cAMP activation alters NF-κB, TNFα, AKAP95, and Cyclin D3 expression, as well as cell viability, in Schwann cells treated with 0.1 (A) and 10 (B) μg/mL of LPS. In cells treated with 0.1 μg/mL of LPS, cAMP did not much of an effect on NF-κB expression but upregulated TNFα, AKAP95, and Cyclin D3 expression, as well as decreased cell viability, compared to the control. On the other hand, in cells treated with 10 μg/mL of LPS, cAMP downregulated NF-κB, AKAP95, and Cyclin D3 expression, while still upregulating TNFα expression and decreasing cell viability, compared to the control.

Conclusions:

- cAMP activation downregulates or has no effect on NF-κB expression in LPS-treated Schwann cells
- cAMP activation upregulates TNFα expression in LPS-treated Schwann cells
- Upregulation of TNFα expression in Schwann cells may be independent of NF-κB pathway
- Decreased viability in LPS-treated Schwann cells may be independent of AKAP95 and cyclin D3

Future Research:

- Immunofluorescence
- Nuclear vs. cytosolic NF-κB expression
- TNF ELISA

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