The Effects of Rolipram, a Selective Phosphodiesterase Inhibitor, on Immortalized Schwann Cell Proliferation

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Introduction: Schwann cells support neurons within the Peripheral Nervous System (PNS) and are responsible for the protection and myelination of the axon. When nervous tissue is damaged within the PNS, Schwann cells will dedifferentiate, proliferate, and migrate towards the site of injury before re-differentiating to form Bunger bands[1]. Due to this capability, Schwann cell transplants have been used in attempt to treat damaged nervous tissue. In the Central Nervous System (CNS), due to inadequate growth and cell signaling factors, there is little success with these transplants[2]. Schwann cell proliferate under *in vitro* conditions when stimulated by the neuronal growth factor heregulin and forskolin a pharmacological activator of cyclic adenosine monophosphate (cAMP)[3]. In recent years, researchers have been using phosphodiesterase inhibitors as an alternative form of treatment for spinal cord damage, Multiple Sclerosis, Alzheimer's Disease, and other neurodegenerative diseases[4]. Phosphodiesterase inhibitors increases the abundance of the universal secondary messenger, cAMP by hydrolyzing a family of enzymes called phosphodiesterases[5]. Intracellular cAMP binds to the regulatory subunit of Protein Kinase A (PKA) which allows the catalytic subunits to phosphorylate protein substrates. PKA is anchored by A kinase-anchoring proteins (AKAPs) that are known to scaffold phosphodiesterases, phosphatases, and other signaling molecules. A critical AKAP that can be found within Schwann cells is ezrin; a protein responsible for the formation of the cytoskeleton, actin filaments, and microvilli as well as serving as a transport linker between the cytoplasm and cell membrane[6]. Currently there is no literature that explores the effects of phosphodiesterase inhibitors such as rolipram on Schwann cells proliferation and ezrin expression.

Our objectives for this research project was:

1. To determine the optimal concentration(s) of rolipram that is required for cell proliferation.
2. To examine the expression of ezrin before and after treating with rolipram.

Morphology of Schwann Cells When Incubated Under Different Mitogenic Conditions:

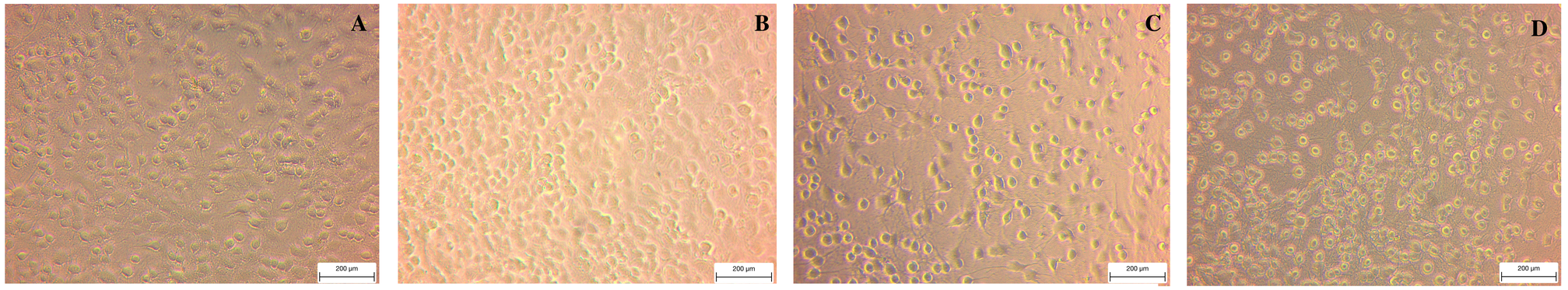

Figure 1: Immortalized Schwann cells were cultured in A) N2 (control) media, B) heregulin (H) 12.5 ng/mL, C) forskolin (F) 1 µM, D) heregulin + forskolin (H+F).

Rolipram Dose Response:

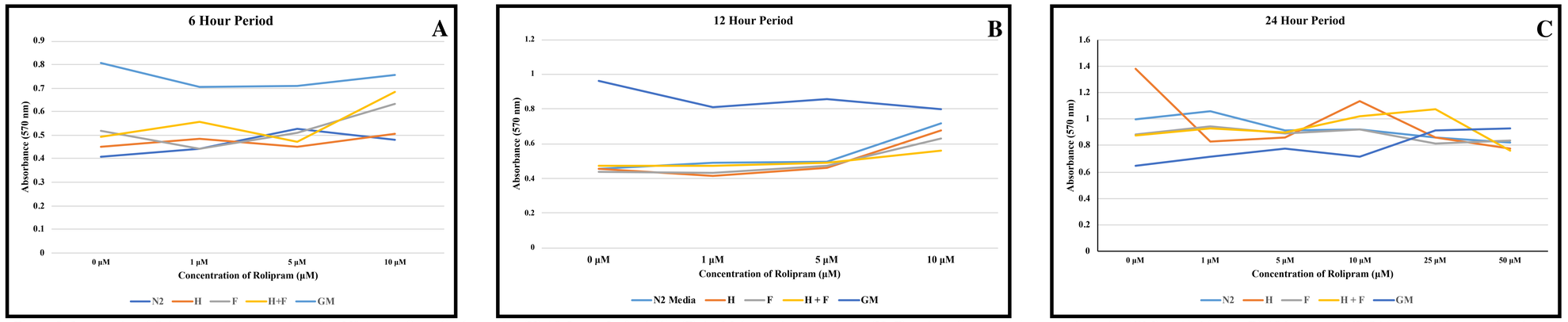

Figure 2: Immortalized Schwann cells were cultured in N2 (control) media, heregulin (H) 12.5 ng/mL, forskolin (F) 1 µM, heregulin + forskolin (H+F) and media containing growth media (GM) at A) 6 hours, B) 12 hours, and C) 24 hours while treated under various concentrations of rolipram (µM). Cell proliferation was measured using MTT Proliferation Assay (n=1).

Ezrin Immunoblot:

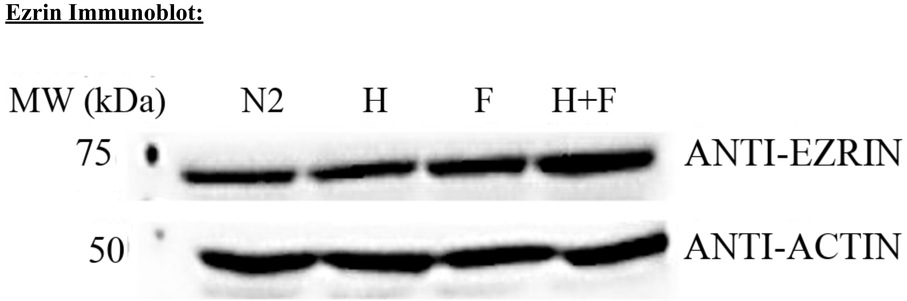

Figure 3: Schwann cells were incubated under N2 (control) media, heregulin (H) 12.5 ng/mL, forskolin (F) 1 µM, or heregulin + forskolin (H+F). The figure above is representation of one immunoblot from three independent experiments.

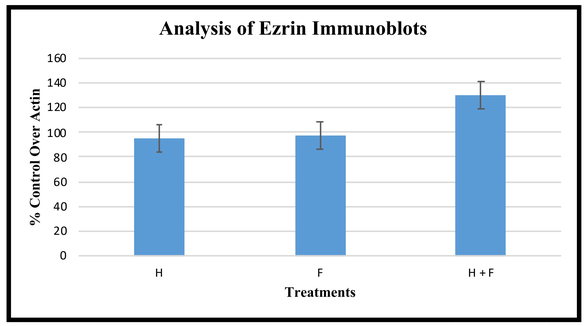

Figure 4: Quantitative expression of ezrin as percent control over actin. Cells treated with heregulin + forskolin had the highest overall ezrin expression (n=3).

Conclusions:

- Cells treated with both heregulin and forskolin appear to be higher in cell density in comparison to N2, heregulin, and foskolin.
- Schwann cell proliferation appears to be optimal at a dose of 0.5 µM and 10 µM rolipram at both 12 and 24 hours time points.
- Expression of ezrin was the highest in cells treated with heregulin and forskolin.

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References:

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