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The Effect of Creatine on Immortalized Schwann Cell Proliferation

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Introduction

Schwann cells support neurons in the peripheral nervous system. They function to myelinate the axons of neurons, which aids in the neuron's speed of conductivity. Schwann cells are also known to aid in the repair of neurons, when myelin is damaged[1]. Previous studies in Schwann cell cultures have shown that addition of heregulin, a neuronal growth factor, and forskolin, a pharmacological activator of cAMP, stimulates a synergistic growth response[2]. Although these growth factors and signaling molecules have been studied in Schwann cell growth, not much is known about creatine, an important component of the phosphocreatine energy buffer system that is crucial for providing ATP during neuronal repair[3]. Based on the significance of creatine in reducing neuronal losses[4], we hypothesized that addition of creatine, with growth factors to Schwann cell cultures will stimulate proliferation. Therefore, the primary objective of this study is to determine the optimal dose and time point, at which creatine stimulates Schwann cell growth in cultures incubated with heregulin and forskolin.

References:

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Creatine Dose Response

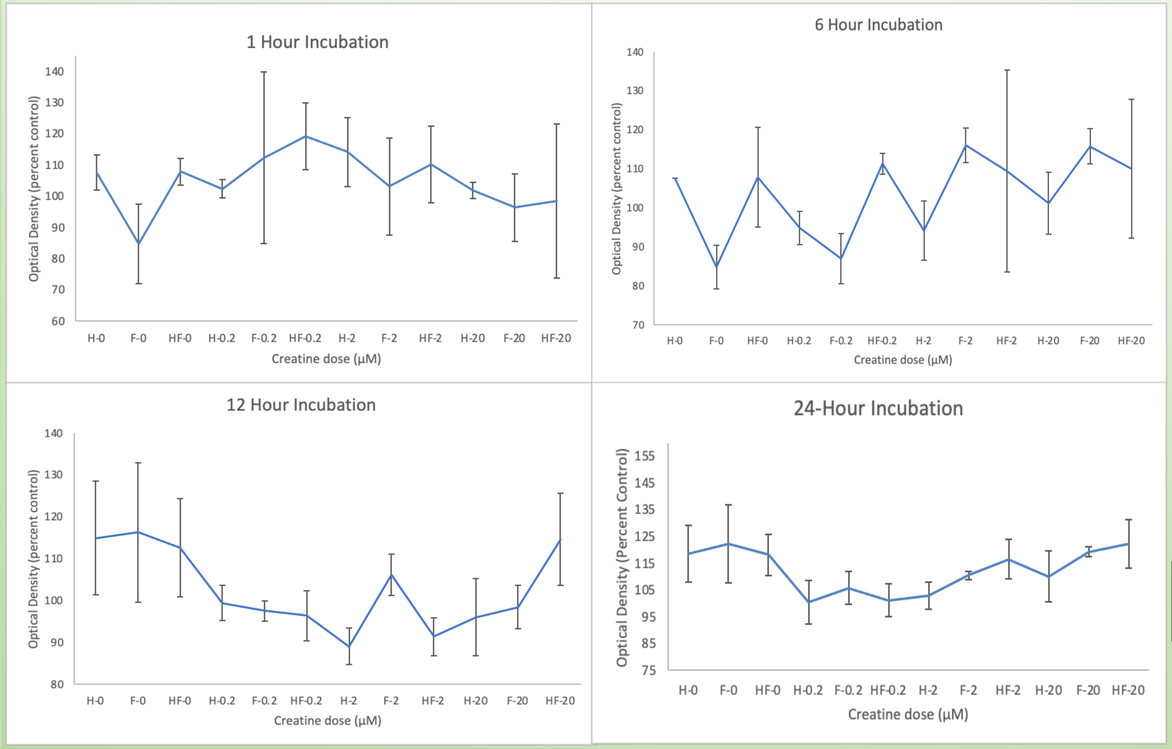

Fig.1 – Effect of creatine on Schwann cell growth: Schwann cells from S16 cell line (SC-2941, ATCC, Manassas, VA) were incubated with N2 (control medium) (0), heregulin (H), forskolin (F), or heregulin + Forskolin (HF) for 1, 6, 12, or 24 hours along with 0, 0.2µm, 2µM or 20µM creatine. To determine cell growth, a colorimetric proliferation assay using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5,-diphenyltetrazolium bromide) (Thermofisher Scientific, Waltham, MA) was performed. MTT was added to the cells for four hours followed by incubation with SDS. Optical density was measured using the Spectramax plate reader (Molecular Devices, San Jose, CA) and analyzed with SoftmaxPro software. The experiment was replicated three times. Using the SPSS software package, data was analyzed by ANOVA. Post hoc tests for comparison between means were further analyzed using Least Significant Difference. A p value < 0.05 was considered statistically significant (Table 1)

Conclusions

1-Hour
- Addition of 0.2µM and 2µM creatine increased proliferation for all treatments in comparison to control.

6-Hour
- Addition of 0.2µM creatine stimulated cell growth in control and heregulin+forskolin treated cultures. Addition of 2µM CR increased growth in cells treated with heregulin and forskolin in comparison to control

12-Hour
- A dose of 0.2µM creatine increased proliferation in control cultures. Forskolin-stimulated cells elicited the highest proliferation at a dose of 0µm of creatine in comparison to control

24-Hour
- Cells treated with both heregulin and forskolin displayed highest proliferation at a dose of 2µM creatine. Cells incubated with 0.2µM and creatine showed a reduction in proliferation for all treatments in comparison to control.

In summary, forskolin increased proliferation at all time points implying that creatine stimulates cAMP-mediated pathways at the 2µM concentration. Addition of both heregulin and forskolin for 24 hours with 2µM creatine resulted in a substantial increase in proliferation. These results suggest that creatine-induced proliferation of Schwann cells appear to be influenced by dose and incubation period.

P-values for different dose and time of Creatine Treatment

Table 1

Time	Dose and Growth Factor	p-value
6 hour	0µM CR F, 2µM CR F	0.029
	0µM CR F, 20µM CR F	0.03
	0.2µM CR F, 20µM CR F	0.043
12 hour	0µM CR H, 2µM CR H	0.027
	0µM CR H, 2µM CR HF	0.042
	0µM CR F, 2µM CR H	0.02
	0µM CR F, 2µM CR HF	0.032
	0µM CR HF, 2µM CR H	0.042
	2µM CR HF, 20µM CR HF	0.045
24 hour	0µM CR N2, 0µM CR F	0.03
	0µM CR N2, 20µM CR HF	0.031
	0µM CR F, 0.2µM CR N2	0.03
	0µM CR F, 0.2µM CR H	0.033
	0µM CR F, 0.2µM CR HF	0.04
	0µM CR F, 2µM CR N2	0.03
	0µM CR F, 20µM CR N2	0.03
	0.2µM CR N2, 20µM CR HF	0.031
	0.2µM CR H, 20µM CR HF	0.034
	0.2µM CR HF, 20µM CR HF	0.04
	2µM CR N2, 20µM CR HF	0.031
	20µM CR N2, 20µM CR HF	0.031