Effects of polyamines and trenbolone acetate on proliferation rates of murine myoblasts

PSIV-9

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Introduction

Androgens have been shown to improve many different aspects of skeletal muscle growth, however, the exact mechanisms through which these molecules improve skeletal muscle growth remains unknown. Research has shown that androgens may modulate the polyamine biosynthetic pathway, resulting in improved skeletal muscle growth (1). Polyamines, found in tomatoes, potatoes, matured cheeses, and most meats, are naturally occurring amino acid derivatives that are known to be modulators of growth (2). As such, we investigated how trenbolone acetate (TBA) and polyamines affect proliferation of murine myoblast cells.

Hypothesis

- Proliferation rate will be increased in murine Sol8 and C2C12 myoblasts when treated with a testosterone analog, TBA, polyamine precursors, or polyamines.

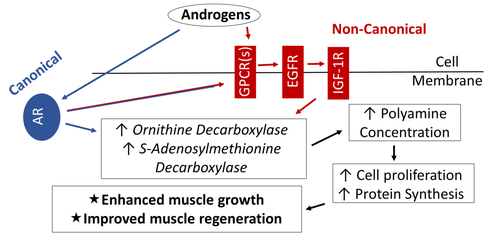

Figure 1. Hypothesized mechanism through which androgens improve skeletal muscle growth involving the polyamine biosynthetic pathway.

Specific Objectives

1. Determine how proliferation rates of murine Sol8 and C2C12 myoblasts are impacted when treated with polyamines or polyamine precursors.
2. Determine how proliferation rates of murine Sol8 and C2C12 myoblasts are impacted when treated with TBA.

Methods

Cell Culture → Treatment → DELFIA Cell Proliferation Assay → Statistical Analysis

- Sol8 and C2C12 mouse myoblast cell lines were obtained from American Type Culture Collections®.
- Cells were cultured in 75 cm^2 flasks containing phenol-red free Dubelcco's Modified Eagle Medium with 10% fetal bovine serum (FBS) and an antibiotic/antimycotic.
- Cells were plated into 96 well plates and allowed 24 hours to establish prior to treatment.

Methods (*continued*)

- Once cultures reached 70% confluency (24 h after plating), cultures were treated with 1% FBS +/- one of the following: 10 nM TBA, 10 mM methionine (Met), 8 mM ornithine (Orn), Mm putrescine (Put), 1.5 mM spermidine (Spd), or 0.1 mM spermine (Spe).
- Proliferation assays were performed 21 h after treatment using a commercially available proliferation kit (Delfia, Perkin Elmer). Results of the proliferation assay were analyzed on a plate reader.
- Assay results were analyzed using the PROC MIXED procedure of SAS with treatment serving as a fixed effect and plate and experiment number serving as random effects. Differences between the least square means of treatments were determined using a Tukey adjustment.

Results

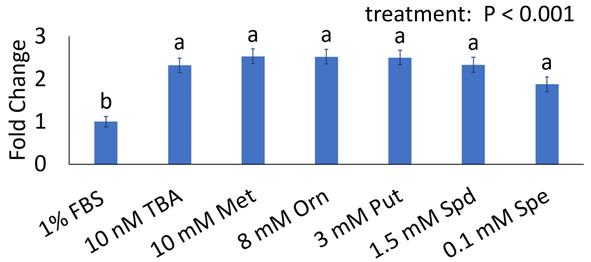

Figure 2. Effects of TBA, polyamines (Put, Spd, and Spe), or polyamine precursors (Met and Orn) on proliferation rate of Sol8 murine myoblast cells. Values represent the fold change relative to the control and the LSMean ± SEM. Bars with different letters indicate a different ($P < 0.05$) proliferation rate when compared to the control treatment (1% FBS).

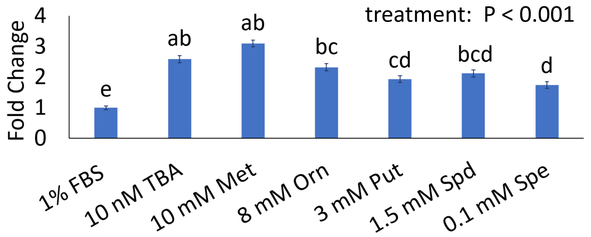

Figure 3. Effects of TBA, polyamines (Put, Spd, and Spe), or polyamine precursors (Met and Orn) on proliferation rate of C2C12 murine myoblast cells. Values represent the fold change relative to the control and the LSMean ± SEM. Bars with different letters indicate a different ($P < 0.05$) proliferation rate when compared to the control treatment (1% FBS).

Conclusions

- In the Sol8 cells, the fold change relative to the control group was increased ($P < .001$) in the 10 nM TBA, 10 mM Met, 8 mM Orn, 3 mM Put, 1.5 mM Spd, and 0.1 mM Spe treatments when compared to the control (1% FBS) cultures.
- In the C2C12 cells, the fold change relative to the control group was increased ($P < .001$) in the 10 nM TBA, 10 mM Met, 8 mM Orn, 3 mM Put, 1.5 mM Spd, and 0.1 mM Spe treatments when compared to the control (1% FBS) cultures.
- These results show that TBA and polyamines and their precursors are capable of increasing murine myoblast proliferation rates.
- **These results suggest that the rate of skeletal muscle growth has the potential to be enhanced when treated with TBA or polyamines and their precursors.**

Future Studies

- We are currently in the process of analyzing mRNA expression of five different genes known to be involved in skeletal muscle growth to determine the mechanisms through which polyamines and their precursors and TBA increase proliferation of murine myoblasts.

Acknowledgements

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References

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