TACSM Abstract

Pharmacological Inhibition of mTOR and ERK1/2 Resulted in Attenuated Protein Synthesis Rates in Differentiated C2C12 Myoblasts in a Similar Fashion to *in vivo R*odent Studies.

J WILLIAM DEAVER, COLLEEN L O'REILLY, STEPHEN F CROUSE FACSM, JAMES D FLUCKEY

Muscle Biology Laboratory; Health and Kinesiology; Texas A&M University; College Station, TX

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Advisor / Mentor: Dr. Fluckey, James (jfluckey@tamu.edu)

ABSTRACT

Fractional protein synthesis rates have long been used as in indicator of acute alterations in the anabolic state of various tissues. Through the use of a number of stable and isotopic tracer methodologies, the measurement of fractional synthesis rates (FSR) *in vivo* has become a staple of skeletal muscle physiology. Through the application of a deuterium oxide tracer, this project sought to measure pharmacological perturbations in fractional synthesis rates *in culture* in differentiated C2C12 murine myotubes.

PURPOSE: To assess myofibrillar protein FSR in differentiated C2C12 murine myotubes following pharmacological inhibition of rapamycin-sensitive (mTOR) or -insensitive (ERK1/2) pathways, and how signal transduction through these pathways impact FSR as compared to previous *in vivo* studies of pharmacological inhibition studies in skeletal muscle.

METHODS: C2C12 murine myoblasts were cultured in collagen coated 6 well culture dishes, and grown to 60-70% confluency using a high glucose DMEM growth media (GM). Cultures were transitioned to a differentiation media (DM) upon reaching target confluency. DM was changed daily for 4 days to allow for complete differentiation to myotubes. Cultures were randomly assigned treatment conditions of cell control (CC), rapamycin inhibition (RAPA), ERK1/2 inhibition (ERK), and electrical stimulation (ESTIM). Cultures underwent treatment conditions for 24 hours with a 4% deuterium oxide GM supplement. Analysis was carried out using a gas chromatography mass spectrometer.

RESULTS: Fractional rates of protein synthesis were significantly lower in the RAPA ($p=0.028$) and ERK ($p=0.029$) groups as compared to CC, with no differences between RAPA and ERK groups ($p>0.05$). Although statistics were not applied to the ESTIM group due to low sample size, electrical pulse stimulation shows promise for the stimulation of FSR in cultured myotubes.

CONCLUSION: Diminished FSR in both RAPA and ERK groups are consistent with previous findings from *in vivo* rodent studies. These results may indicate comparable alterations in skeletal muscle anabolic signaling in cell culture as well as *in vivo* rodent models. Further investigations into anabolic signaling mechanisms related to the control of protein synthesis are needed.