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

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Fractional protein synthesis rates have long been used as an 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.