TACSM Abstract

Insulin-induced Increase in Anabolic Capacity is Blunted by Autophagic Inhibition in L6 Myotubes

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ABSTRACT

Insulin is an anabolic hormone that acts on skeletal muscle cells to stimulate protein synthesis, an effect that is enhanced by the availability of amino acids. While autophagy within the cell provides an intracellular source of amino acids to support anabolism, little is known about how this pathway impacts the insulin-induced increase in anabolic capacity within skeletal muscle cells. **PURPOSE:** The purpose of this study was to determine the impact of autophagic inhibition in cultured L6 myotubes in conjunction with insulin stimulation in vitro. **METHODS:** Differentiated, cultured L6 myotubes were treated for 24 hours with or without insulin (100 nM) and NSC 185058 (100 µM), a specialized inhibitor of the autophagic catabolic pathway, in media enriched with 4% deuterium. Cells were harvested from each treatment group (n=3/group) 24 hours post-deuterium enrichment and were processed for protein synthesis and western blot protein analysis. A one-way ANOVA was used to compare groups, and when significant F ratios were present, a Student's Newman-Keuls post hoc procedure was used to test differences among group means. Alpha was set at $p \leq 0.05$ for all analyses. **RESULTS:** Cells treated with insulin (INS) had a higher ratio of phosphorylated to total P70S6K compared to untreated (CON) cells and those incubated with both insulin and NSC 185058 (INS+NSC; 1694% and 327%, respectively; $p<0.05$). INS+NSC also decreased the ratio of phosphorylated to total 4EBP1 relative to CON (-51%) and INS (-49%), although these differences were not significant ($p>0.05$). Myofibrillar protein synthesis was stimulated with INS compared to CON and INS+NSC (30.3% and 70.1% respectively; $p<0.05$) but was lower in INS+NSC relative to CON (-23.4%; $p<0.05$). **CONCLUSION:** Results from our study indicate that insulin (100 nM) stimulates anabolism in skeletal muscle cells, but that addition of the autophagic inhibitor NSC 185058 (100 µM) blunts this effect to a level similar to or less than control. Further, our data suggest that the reduction of protein synthesis is mediated through the downregulation of the mTORC1 signaling pathway. While it is widely recognized that insulin promotes anabolic activity through both the direct stimulation of mTOR signaling and extracellular amino acid uptake, our data strongly indicate that autophagic processes are necessary for full anabolic responses in muscle. This decrease in anabolic capacity supports previous literature indicating that the amino acid availability impacts the stimulatory impact of insulin on protein synthesis.