TACSM Abstract

Autophagy is Required for mTOR-Mediated Anabolism in Skeletal Muscle

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ABSTRACT

PURPOSE: While much has been discovered about the role autophagy in protein degradation, recent evidence suggests that autophagy is required for muscular adaptations to exercise, hinting at a hitherto unknown cross-talk between autophagic proteolysis and muscle protein anabolism. Here, we set out to further elucidate the metabolic mechanisms by which autophagy may influence protein anabolism. **METHODS:** L6 myoblasts received either electrical pulse stimulation (EPS) to induce muscle contraction or were unstimulated to serve as controls, and were then treated with an inhibitor of the ATG4 enzyme which catalyzes the initial step of autophagy NSC185058 (NSC, 100 μM) or DMSO as a vehicle control (VC). After 24 hours, cells were lysed and Western immunoblotted for P70S6K, DEPTOR, MAPK, AMPK, LC3, and P62. Differences between VC and NSC treated groups were assessed by a two-tailed t-test, while comparisons between VC, EPS, and EPS+NSC groups were made using one-way ANOVA and SNK post-hoc test, with α levels set at 0.05. **RESULTS:** EPS induced a 97% increase in P70S6K phosphorylation ($p<0.05$), with NSC treatment blunting this effect, leading to a 22% increase ($P>0.05$). EPS resulted in a 37% reduction in DEPTOR content ($p<0.05$); however, NSC treatment alone produced a 166% decrease in DEPTOR level ($p<0.05$), with EPS+NSC leading to an even larger reduction (-766%) in DEPTOR than EPS alone. NSC treatment led to a decrease (-85%, $p>0.05$) LC3II/I ratio relative to VC, which was reduced in both the EPS (-68%, $p<0.05$) and EPS+NSC (-87%, $p<0.05$) conditions. P62 content increased by 749% with EPS ($p<0.05$), with no significant difference in P62 level between VC and EPS+NSC, and NSC treatment alone led to a 61% decrease in P62 ($p<0.05$). MAPK phosphorylation was elevated in both EPS (99.9%, $p>0.05$) and EPS+NSC (149.13, $p<0.05$). Neither NSC nor EPS+NSC altered phosphorylation status of AMPK. **CONCLUSION:** Despite reductions in DEPTOR, mTOR activity was blunted in EPS+NSC cells, indicating that mTOR mediated anabolic signaling requires autophagy post muscle contraction. This is particular to the mTOR pathway, as an increase in MAPK phosphorylation was still observed in EPS+NSC. While the decrease in LC3II/I ratio and accumulation of P62 seen after EPS are likely due to inhibition of autophagy due to mTOR activity, our data indicate that inhibition of ATG4 by NSC185058 blunts mTOR activity after muscle contraction. This effect is not due to activation of the cellular energy sensor AMPK, as we found no increase in AMPK phosphorylation in any condition. Further work will be required to fully elucidate the mechanism by which NSC185058 inhibits mTOR-mediated anabolism.