Nτ-methylhistidine decreases myofibrillar protein levels by up- regulating ubiquitin ligases in C2C12 myotubes

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Objectives: Skeletal muscle mass is controlled through a delicate balance between protein synthesis and degradation. Elucidation of the mechanism of protein synthesis and degradation is important for proper control of skeletal muscle mass. N^{τ}-methylhistidine (N^{τ}-MeHis) is formed by post-translational methylation of the histidine residue involved in myosin and actin, which occupy a large part of myofibrillar proteins. Since this amino acid has been considered to be rapidly effused from skeletal muscle, its biological activity in skeletal muscles remains unclear. However, considerable amount of intercellular free N^{τ}-MeHis was actually detected in skeletal muscle. Further, we confirmed that there are individual differences in intercellular free N^{τ}- MeHis concentration of the skeletal muscle even among same-strain broiler chickens. In this study, to investigate biological roles of N^{τ}-MeHis in skeletal muscle, we examined the effects of medium supplementation with N^{τ}-MeHis on myofibrillar protein levels in myotubes.

Materials and Methods: Murine myoblasts (C2C12) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (P/S) for 3 days. Cells were grown in 10 cm dish (for measurement of protein content), 6-well plate (for measurement of protein expression levels) and 12-well plate (for measurement of mRNA expres- sion levels) at 37 °C in 5% (v/v) CO^2 in a humidified environment. To induce myotube formation, the medium was replaced with DMEM supplemented with 2% horse serum and 1% P/S for 5 days. N^{τ}-MeHis was made up in phosphate-buffered saline (PBS) and subsequently added to the appropriate tissue culture wells (1 : 1000). The vehicle-control wells received PBS in the same vol- ume as the treatment groups. C2C12 myotubes were incubated with N^{τ}-MeHis for 48 h in DMEM. We determined total protein content and myofibrillar protein content by Bradford protein assay, mRNA expression levels by quantitative real-time PCR, and protein expression levels by immunoblotting.

Results and Discussion: We examined whether N^{τ}-MeHis supplementation affects the protein content of C2C12 myotubes, and found that the total protein content of C2C12 myotubes was significantly decreased by N^{τ}-MeHis (10 μmol/L). This result indicates that supplementation of N^{τ}-MeHis suppresses muscle growth in C2C12 myotubes. Then, we examined whether N^{τ}-MeHis supple- mentation suppresses myofibrillar protein levels. N^{τ}-MeHis supplementation significantly decreased the myofibrillar protein con- tent, especially MHCII and α-actin protein levels. However, interestingly, it affected gene expression encoding neither MHC nor α- actin mRNA. These results suggest that N^{τ}-MeHis might be involved in the posttranslational degradation of myofibrillar proteins rather than their transcription. It has been well known that two major protein degradation pathways, the ubiquitin (Ub)-proteasome and the autophagic-lysosome pathways, play dominant roles in control of skeletal muscle mass. In this study, we found that N^{τ}- MeHis supplementation significantly increased the mRNA expression of Atrogin-1/MAFbx and MuRF1 which is the muscle-specific ubiquitin ligases. In addition, Atrogin-1/MAFbx protein level was significantly increased in C2C12 myotubes supplemented N^{τ}-MeHis. Since the mRNA expression of Ub ligases clearly correlates with polyubiquitination, they are considered to play an important role in controlling polyubiquitination, a rate-limiting step in the Ub-proteasome system. Therefore, these results suggest that N^{τ}-MeHis supplementation activates the Ub-proteasome pathway. Moreover, we found that N^{τ}-MeHis supplementation also in-creased the mRNA expressions of autophagyrelated factors (Beclin-1 and LC3B). Because the ratio of LC3B-II to LC3B-I is con- sidered as an index for autophagy activity, we then detected LC3B-II/I ratio in C2C12 myotubes. N^{τ}-MeHis supplementation led to a significant increase in the expression of both LC3B-I and LC3B-II. However, since the ratio of LC3B-II to LC3B-I was un- changed by supplementation of N^{τ}-MeHis, we cannot infer whether N^{τ}-MeHis supplementation affected autophagy activity of C2C12 myotubes.

Conclusions: In summary, medium supplementation of N^{τ}-MeHis decreased myofibrillar protein levels via up-regulating the Ub-li- gases in C2C12 myotubes. However, how N^{τ}-MeHis acts on myofibrillar protein in C2C12 myotubes remains unclear. Further in- vestigation is needed to gain insight into the mechanisms by which N^{τ}-MeHis induces protein degradation in myotube.

Key words: Nτ-methylhistidine, Protein Degradation