APOPTOSIS IN UNLOADED OR AGED SKELETAL MUSCLE ARE REGULATED VIA DIFFERENTIAL PATHWAYS

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Apoptosis has function to eliminate myonuclear and satellite cell by DNA fragmentation in atrophied skeletal muscle with aging or by unloading. It is suggested that there are several pathways and a lot of proteins that are related to apoptosis, and the mechanisms for apoptosis in atrophied skeletal muscle are unclear.

PURPOSE: The present study is aimed to determine effector proteins and the induction pathways for apoptosis on atrophying skeletal muscle induced by hindlimb unloading or senescence. We hypothesized that differential mechanisms were existed to induce the apoptotic atrophy in skeletal muscle between unloading and aging.

METHODS: Male Fischer-344xBrown Norway rats were assigned three groups: 6 month old control (6-mo; n=6), 6-mo hindlimb unloading (6-mo-HU; n=5) and 32-mo old senescence (32-mo; n=5). Hindlimb unloading was performed by tail suspension for 2 weeks. By using in situ TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (UTP)-biotin nick end-labeling) staining, the apoptotic nuclei were examined histochemically. Localization of cleaved-caspase 3, active form of caspase-3 for DNA fragmentation, and cleaved-PARP, one of the down stream targets of active caspase-3, were also analyzed. To investigate the molecular mechanisms responsible for induction of apoptosis, we analyzed the expression level of proteins related to mitochondrial and endoplasmic reticulum pathways in unloaded and aged muscles by western blotting.

RESULT: Absolute soleus muscle weights in 6-mo-HU and 32-mo rats were significantly lower than that in 6-mo control, indicating unloading-or aging-induced atrophy. TUNEL positive nuclei were identified as myonuclei or satellite cells which exist inside of muscle fiber basal lamina in both atrophied soleus muscles, but hardly identified in 6-mo control. Cleaved caspase-3- and PARP-positive nuclei were observed in 32-mo senescent rats, while those nuclei were hardly identified in 6-mo control and 6-mo-HU rats. Analyses of westernblots from mitochondrial and cytosolic extracts also showed differential expression patterns between unloaded and aged muscles. In mitochondrial fraction, Bcl-2, a mitochondrial apoptotic regulator, was significantly decreased in 6-mo-HU, while increased in 32-mo. Caspase-12 and CHOP/GADD153, endoplasmic stress-inducible apoptotic regulator, were markedly increased in cytosolic fraction of 32-mo soleus, but unchanged in 6-mo-HU. These results suggested that the key factors for apoptosis induction were the decrease of Bcl-2 on mitochondria in unloaded muscle and the increase of endoplasmic stress, such as caspase-12 or CHOP/GADD153, on cytosol in aged muscle.

CONCLUSION: The present data provide new evidence that differential mechanisms regulate the induction of apoptosis between unloaded and aged skeletal muscles.

Keywords: Apoptosis, Atrophy, Skeletal Muscle