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The molecular basis of skeletal muscle atrophy – parallels with osteoporotic signaling

S. Kandarian

Department of Health Sciences, Boston University, Boston, MA, USA

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Multiple triggers can lead to concomitant loss of bone and muscle including reduced biomechanical loading, aging, and systemic inflammatory conditions (e.g., cancer, rheumatoid arthritis, etc.). Given the *in vivo* physiological interdependence and the developmental relationships between muscle and bone it is not surprising to find at least one pathway that regulates both bone and muscle loss. It appears that nuclear factor of kappaB (NF-kB) signaling is involved in these processes in both tissue types in cachectic conditions[1,2] and this is also the case with aging and with reduced mechanical loading[3,4]. The focus of this presentation is to summarize the role of NFkB proteins and their immediate upstream regulators in muscle wasting due to short-term hindlimb unloading in rodents.

Nuclear factor of kappaB (NF-kB) is a family of 5 (mammalian) transcription factors that contain a Rel homology domain. This domain allows the transcription factors to form dimers, bind to a specific DNA consensus sequence, and bind to a cytosolic docking protein called inhibitor of kappaB (IkB). In 2002 we showed that 7 days of hindlimb unloading leads to atrophy and a robust activation of NF-kB activity in the soleus muscle but not in the extensor digitorum longus muscle which does not atrophy at 7 days of unloading[5]. Activation of NF-kB is induced as early as 3 days of unloading[6] and is sustained until at least 10 days[7] and thus is not transient. Plantaris muscles also show unloading-induced atrophy and activation of NF-kB. Inflammation does not appear to accompany muscle unloading since neither AP-1 nor NFAT activity is activated[5]. JNK activity and TNF levels are unchanged in unloaded muscles further supporting a lack of an inflammatory response. Lastly, there is no evidence of complement activation in unloaded muscles[8]. Results from immunoblotting of nuclear extracts and gel supershift assays suggested that the Rel proteins p50 and c-Rel could be involved in the activation of NF-kB, as well as an IkB-like family member which acts as a co-transactivator called Bcl-3. Knockout mice for each one of these three genes showed that p50 and Bcl-3 were required for the unloading-induced activation of NF-kB and muscle atrophy[7], but c-Rel was not[6]. Using mouse Affymetrix microarrays, we have been able to identify potential p50 and Bcl-3 target genes by comparing the response to unloading in muscles from wild type vs. p50 or bcl3 knockout mice (unpublished data). As expected more genes were upregulated by muscle unloading in wild type vs. knockout mice since the muscles from knockout mice atrophy only very minimally. Thus in knockout mice, the lack of change in a gene normally upregulated by unloading suggests that it is a p50 or Bcl-3 target gene.

The major upstream regulator of Rel proteins, IkBalpha, was then studied for its involvement in atrophy. Because knockout mice are embryonic lethal, we overexpressed a form of IkBalpha, which acts to inhibit classical NF-kB signaling; this mutant protein is called the IkB super repressor. It is missing amino acids 1-36 so it cannot be phosphorylated by upstream NF-kB kinases thereby blocking its degradation by the proteasome and blocking its release of bound Rel dimers which would otherwise localize to the nucleus to activate NF-kB target genes. Gene transfer of the IkB super repressor, fused to EGFP, into rat soleus muscles resulted in a 40% inhibition of muscle atrophy and a complete inhibition of NF-kB reporter gene due to 7 days of unloading[6]. The unloading-induced increase in ubiquitinated protein was

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Corresponding author: Susan Kandarian Ph.D., Department of Health Sciences, Boston University, 635 Commonwealth Ave, Room 44, Boston, MA 2215, USA
E-mail: skandar@bu.edu

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also inhibited by expression of the super repressor. Thus IkB alpha appears to have a role in unloading-induced muscle wasting.

The inhibitor of kappaB kinase (IKK) phosphorylates IkB and is comprised of a complex of two kinases, IKKalpha and IKKbeta, and a regulatory subunit called NF-kB essential modulator (NEMO). Constitutively active (c.a.) IKKbeta has been shown to induce muscle wasting in transgenic mice showing that it is sufficient to elicit atrophy[2]. We showed that overexpression of a dominant negative (d.n.) form of IKKbeta (fused to EGFP) blocked 7 day unloading atrophy in the soleus by 50% demonstrating its requirement in the atrophy process[9]. The same results were shown with overexpression of d.n.IKKalpha. In addition we showed that overexpression of d.n.IKKalpha plus d.n.IKKalpha had a partially additive effect on atrophy inhibition (70%) suggesting that these two kinases may not have identical substrates. Overexpression of d.n.IKKalpha or d.n.IKKbeta completely inhibited the unloading-induced activation of the NF-kB dependent reporter gene. Finally, c.a. IKKalpha was also sufficient to elicit atrophy and activate the NF-kB reporter gene in muscle after 7 days of overexpression[9]. These data demonstrate that multiple components of NF-kB signaling are either necessary and/or sufficient in regulating the loss of muscle protein. Ongoing experiments will identify the target genes that carry out the muscle wasting process.

The role of NF-kB signaling proteins in disease-induced muscle wasting is also apparent but not as many of the component proteins have been identified[2]. However, in this case NF-kB activation appears to be associated with inflammatory processes and activation of cytokines such as TNF[2]. This is similar to systemic inflammatory conditions that affect bone resorption via the action of NF-kB signaling[1]. In fact, the IkB super repressor has been shown to block inflammatory arthritis and bone loss[10]. Also inhibition of IKKbeta by a decoy peptide blocks osteoclast activity and bone erosion in inflammatory arthritis[11,12]. Therefore it appears that there is overlap in the NF-kB regulation is operative in both bone loss and muscle atrophy. This is logical given the functional interaction of these tissues. More work is needed to identify how NF-kB can regulate muscle and bone loss in the presence and absence of inflammation, perhaps by comparing the target genes that are activated in each case.

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