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Stress and Muscle Cachexia

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Abstract

One of the metabolic hallmarks of sepsis and severe injury is muscle cachexia, mainly reflecting increased protein breakdown, in particular myofibrillar protein breakdown. This review describes recent knowledge regarding the molecular mechanisms of sepsis-induced muscle cachexia. Among different intracellular proteolytic pathways, the energy-ubiquitin-dependent pathway is particularly important for the regulation of muscle protein breakdown during sepsis, both in animals and humans. The gene expression of ubiquitin, ubiquitin-conjugating enzyme $E2_{14k}$, ubiquitin ligase E3α and several components of the 20S proteasome is upregulated and the activity of the 20S proteasome is increased in septic muscle. In addition, sepsis-induced muscle proteolysis can be blocked by specific proteasome inhibitors both in vivo and in vitro. Sepsis is also associated with increased calcium levels and upregulated gene expression of calpains in skeletal muscle. Calcium-calpain-dependent release of myofilaments from the sarcomere provides substrates for the ubiquitin-proteasome pathway and may be an early, perhaps rate-limiting component of sepsis-induced muscle cachexia.

Key Words: Muscle; Sepsis; Proteolysis; Ubiquitin; Proteasome; Calpain.

Introduction

Sepsis and severe injury, including burn injury, are associated with increased degradation of muscle proteins, resulting in muscle wasting (Hasselgren, 1999a). There is evidence that muscle protein breakdown in these conditions mainly reflects degradation of the contractile myofibrillar proteins actin and myosin (Hasselgren et al., 1989), in part explaining why patients with severe metabolic stress experience muscle fatigue. Muscle cachexia in patients with sepsis and severe injury has important clinical implications because it can prevent or delay ambulation, increasing the risk for thromboembolic complications and prolonging rehabilitation. In addition, when respiratory muscles are affected (Reid and MacGowan, 1998), there is an increased risk for pulmonary complications and a need for extended ventilatory support. Understanding the mechanisms, including the molecular regulation, of muscle cachexia therefore is important both from a biological and clinical standpoint. The purpose of this review is to describe recent knowledge with regards to intracellular mechanisms of sepsis-induced muscle proteolysis. In particular, evidence for involvement of the ubiquitin-proteasome and calcium-calpain-dependent proteolytic pathways is discussed. More extensive reviews of intracellular mechanisms and molecular regulation of muscle cachexia were recently published elsewhere (Mitch and Goldberg, 1996; Hasselgren and Fischer, 2001).

The Ubiquitin-Proteasome Pathway

The ubiquitin-proteasome proteolytic pathway was reviewed recently by Hershko and Ciechanover (1998) and will be described only briefly here. Proteins degraded by this mechanism are first conjugated to multiple molecules of ubiquitin which is a 76 amino acid, 8.5 kDa residue. The ubiquitination of proteins is regulated by at least three sets of enzymes, i.e., the ubiquitin activating enzyme E1, ubiquitin conjugating enzyme E2, and ubiquitin ligase E3. The structure of the ubiquitin system is heiarchial in that a single E1 activates ubiquitin for most modifications whereas substrate and tissue specificity are accounted for by different E2s and E3s. There is evidence that the 14 kDa $E2_{14k}$ and E3α are particularly important for the degradation of proteins in skeletal muscle (Wing and Banville, 1994; Kwon et al., 1998).

After ubiquitination, proteins are recognized and degraded by the 26S proteasome. The 26S proteasome is a large proteolytic complex consisting of the 20S proteasome, in which the actual degradation of proteins occur, and 19S capping proteins attached to each end of the barrel-shaped 20S proteasome. The 19S complex accounts for recognition, binding and unfolding of the ubiquitinated protein which after its unfolding is funneled through the central channel of the 20S proteasome and hydrolyzed. A simplified scheme of the ubiquitin-proteasome pathway and the different steps involved in the ubiquitination of proteins are shown in Fig 1.

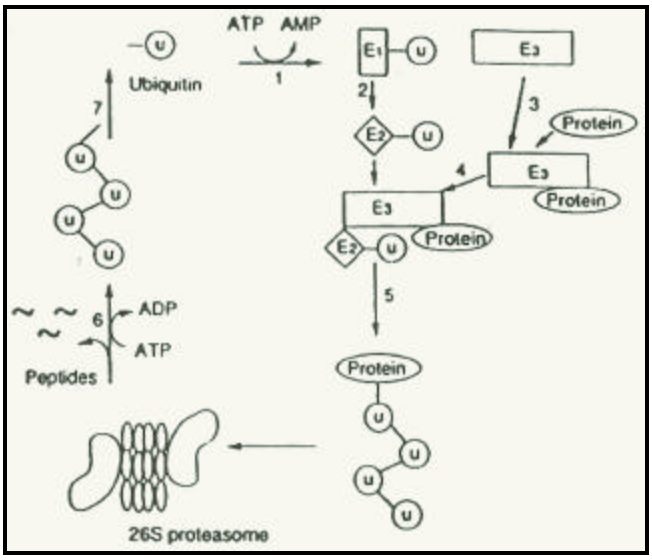

Figure 1. Simplified scheme of the ubiquitin-proteasome proteolytic pathway. In this pathway, ubiquitinated proteins are recognized and degraded by the 26S proteasome. The steps involved in the breakdown of proteins by this mechanisms include 1) activation of ubiquitin by the ubiquitin activating enzyme E1; 2) transfer of ubiquitin to the ubiquitin conjugating enzyme E2; 3) interaction between the substrate protein and the ubiquitin ligase E3; 4) interaction between E2 and E3 resulting in 5) multiubiquitination of the substrate protein; 6) degradation of the ubiquitinated protein by the 26S proteasome; and 7) deubiquitination resulting in the release and reuse of ubiquitin in the pathway. Energy is required for at least two steps in the pathway; activation of ubiquitin by E1 (step 1) and the proteolytic activity in the 26S proteasome (step 6). (From Hershko, 1996, with permission).

There are several lines of evidence supporting a role of ubiquitin-proteasome-dependent proteolysis in muscle cachexia caused by sepsis (Hasselgren, 1999a; Hasselgren and Fischer, 2001) and other catabolic conditions as well, including burn injury (Fang et al., 1995, 1998a), starvation (Wing and Goldberg, 1993), uremia (Price et al., 1994), AIDS (Llovera et al., 1998), and cancer (Baracos et al., 1995; Williams et al., 1999b). In studies in our laboratory, the gene expression of ubiquitin and several of the 20S proteasome subunits was upregulated in muscles from rats with sepsis (Tiao et al., 1994; Hobler et al., 1999a,b). Because the stability of ubiquitin mRNA in skeletal muscle was not influenced by sepsis, the increased ubiquitin mRNA levels most likely reflected increased transcription of the ubiquitin gene in septic muscle (Tiao et al., 1997b). Evidence for increased transcription of genes in the ubiquitin-proteasome pathway was reported in other conditions characterized by muscle wasting as well (Bailey et al., 1996). In additional studies, we found that mRNA levels for ubiquitin and the proteasome subunit C3 were increased in muscle from patients with sepsis, suggesting that muscle cachexia is regulated by the ubiquitin-proteasome pathway also in humans (Tiao et al., 1997a). In addition to ubiquitin and several proteasome subunits, sepsis also upregulates the gene expression of the ubiquitin-conjugating enzyme $E2_{14k}$ and the ubiquitin ligase E3α in skeletal muscle (Hobler et al, 1999a; Fischer et al, 2000b). These enzymes work in concert in skeletal muscle and regulate the ubiquitination and degradation of proteins with a destabilizing N-end, the so called N-end rule pathway (Varshawsky, 1997; Kwon et al., 1998). Studies in other laboratories suggest that the activity of these enzymes is increased in atrophying muscles (Solomon et al., 1998a,b). Surprisingly, $E2_{14k}$ protein levels were not increased in septic muscle, despite increased mRNA levels (Hobler et al., 1999a). The reason for this observation is not known at present but may reflect an increased turnover (i.e., an increase in both synthesis and degradation) of $E2_{14k}$ in septic muscle. Interestingly, similar results of increased $E2_{14k}$ mRNA levels with unchanged $E2_{14k}$ protein levels were seen in muscles from burned rats (Fang et al., 2000).

Further evidence for a role of the ubiquitin-proteasome pathway in sepsis-induced muscle cachexia was found in experiments in which the increase in muscle protein breakdown in septic muscle was blocked by specific proteasome inhibitors. Thus, when muscles from septic rats were incubated in the presence of LLnL or lactacystin, both total and myofibrillar protein breakdown rates were reduced to control levels (Tawa et al., 1997; Hobler et al., 1998). A similar effect of proteasome inhibitors was seen in cachectic muscles from burned rats (Fang et al., 1998b). In a

more recent study from our laboratory, treatment of septic rats in vivo with the proteasome inhibitor PSI reduced muscle protein degradation in a dose-dependent manner (Fischer et al., 2000a). Although these results are important by providing further support for a role of the ubiquitin-proteasome pathway in sepsis-induced muscle cachexia, the clinical implications remain unclear at present. Because ubiquitin-dependent proteolysis is involved in the regulation of a large number of cellular functions in addition to muscle protein breakdown, such as regulation of the cell cycle, activation of transcription factors, and the immune response (Hershko and Ciechanover, 1998), the administration of a proteasome inhibitor in vivo may result in significant side effects. Further studies are needed before proteasome inhibitors would be considered for the prevention or treatment of muscle cachexia in patients with metabolic stress.

Calcium/Calpain-Dependent Release of Myofilaments

Because previous studies suggest that it is mainly myofibrillar proteins that are subjected to ubiquitin-proteasome-dependent degradation in cachectic muscle (Hasselgren et al., 1989; Tiao et al., 1994), it is somewhat surprising that the proteasome does not degrade intact myofibrils (Koohmaraie, 1992; Solomon and Goldberg, 1996). Recent observations suggest that actin and myosin are released from the sarcomere by a calcium/calpain-dependent mechanism before ubiquitination and degradation and it is possible that the released myofilaments have destabilizing N-ends and therefore are substrates in the N-end rule pathway. In studies from our laboratory we found morphological evidence for disruption of sarcomeric Z-disks (which are the anchoring sites for actin and myosin) and biochemical evidence for release of myofilaments from the sarcomere (Williams et al., 1999a). In the same study, sepsis resulted in upregulated gene expression of m- and u-calpain and the muscle specific calpain p94. In other experiments, dantrolene, which blocks the release of calcium from intracellular stores (Fruen et al., 1997), prevented the sepsis-induced increase in muscle calcium levels, release of myofilaments and protein degradation (Fischer et al., 2001). Taken together, these observations are consistent with a model of sepsis-induced muscle cachexia in which calcium/calpain-dependent release of actin and myosin is a “proximal” (and perhaps rate-limiting) event followed by ubiquitin-proteasome-

dependent degradation of the myofilaments (Fig 2). If this model is correct, the ubiquitin-proteasome-dependent proteolysis in cachectic muscle may be the response to an increased amount of substrates (released myofilaments) rather than the cause of muscle cachexia.

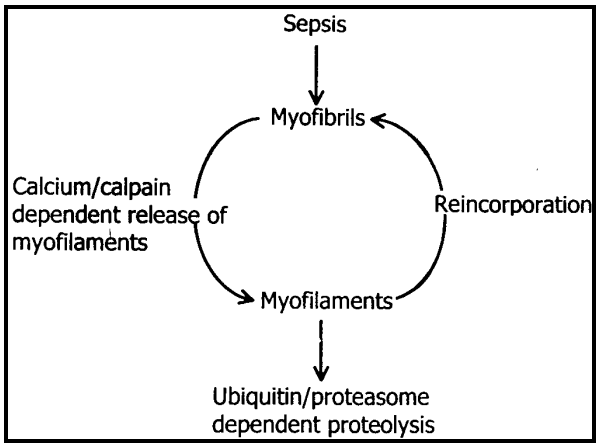

Figure 2. Model of sepsis-induced muscle cachexia. In this model, sepsis results in calcium/calpain-dependent release of myofilaments from the sarcomere. The myofilaments are ubiquitinated in the N-end rule pathway and degraded by the 26S proteasome or reincorporated into the myofibrils. This model offers two levels of possible therapeutic intervention: inhibition of the calcium/calpain-dependent release of myofilaments (e.g., with dantrolene) or inhibition of the ubiquitin-proteasome pathway (e.g., with proteasome inhibitor). (From Hasselgren and Fischer, 2001, with permission).

It is interesting to note that in early studies, the role of the ubiquitin-proteasome pathway was believed to be the breakdown and disposal of abnormal and potentially harmful proteins (Hershko and Ciechanover, 1998). It may therefore be argued that inhibition of the proteasome in cachectic muscle would be harmful, allowing for the accumulation of abnormal proteins. However, other studies (van der Westhuyzen et al., 1981) suggest that myofilaments released from the sarcomere can be reincorporated into the myofibril (see Fig 2) and it is possible that this process is enhanced if the proteasome-dependent degradation of the myofilaments is blocked. More studies are needed to define the relationship between calpain-dependent release of myofilaments and ubiquitin-proteasome-dependent protein degradation in cachectic muscle.

Transcription Factors

Despite the fact that the expression of several genes involved in different proteolytic mechanisms is increased in cachectic muscle, surprisingly little information is available regarding transcription factors in skeletal muscle. In a recent study, we found evidence that sepsis is associated with altered DNA binding activity of the "inflammatory" transcription factors NF-κB and AP-1 (Penner et al., 2001). Interestingly, whereas AP-1 activity was upregulated throughout the septic course in rats after cecal ligation and puncture, NF-κB DNA binding activity was increased early during sepsis but was subsequently downregulated (Fig 3). In other experiments, the DNA binding activity of C/EBP as well was increased in septic muscle (Gang et al., 2000), further supporting the concept that multiple transcription factors that have been found previously to be involved in the inflammatory response in other tissues and cell types are activated in skeletal muscle during sepsis.

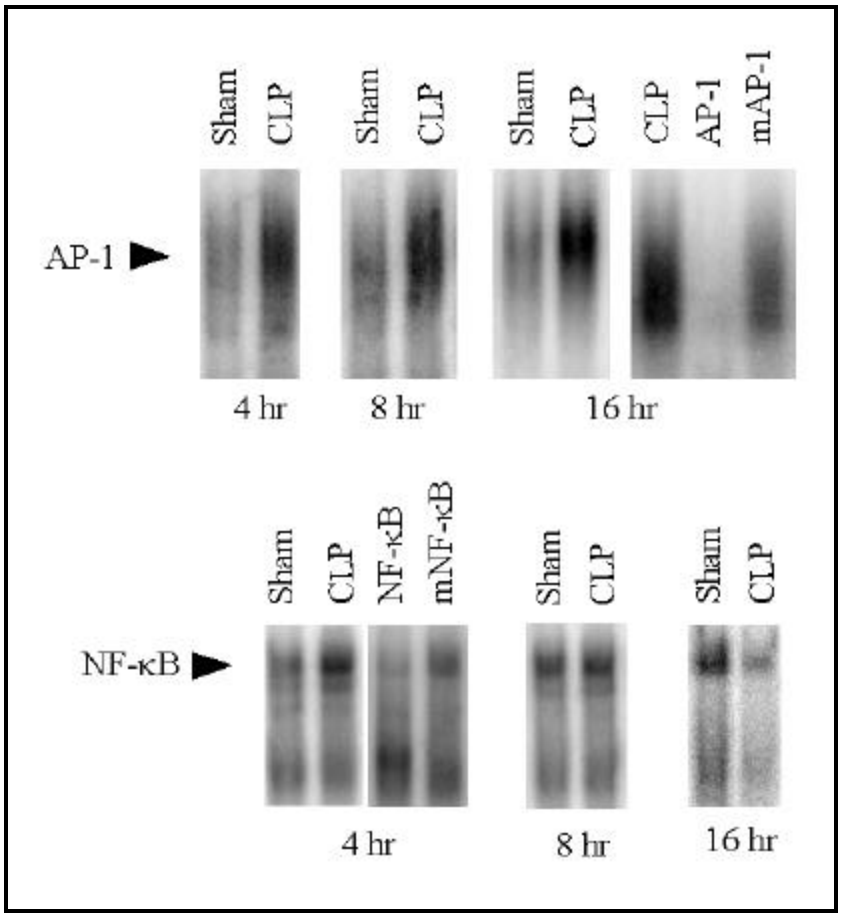

Figure 3. DNA binding activity of the transcription factors AP-1 (upper panel) and NF-κB (lower panel) in rat extensor digitorum longus muscles at various time-points after sham-operation or cecal ligation and puncture (CLP). Competition reactions were performed by adding an excess of unlabeled wild-type or mutant oligonucleotide to the reactions (lanes 8 and 9, upper panel, and lanes 3 and 4, lower panel). (From Penner et al., 2001, with permission).

Glucocorticoids are the most important mediator of sepsis-induced muscle proteolysis (Hasselgren, 1999b). Treatment of septic rats with the glucocorticoid receptor antagonist RU 38486 blocked sepsis-induced muscle proteolysis in previous reports from our laboratory (Hall-Angerås et al., 1991; Tiao et al., 1996). In a more recent study, the same treatment prevented the early up-regulation and the subsequent down-regulation of NF-κB in skeletal muscle (Penner et al., 2001). Taken together, these observations suggest that NF-κB may regulate genes that are involved in the development of muscle cachexia during sepsis. Results from recent in vitro experiments support the concept that NF-κB regulates gene(s) that are involved in the regulation of muscle protein breakdown. Thus, in a study by Du et al. (2000), treatment of cultured myotubes with dexamethasone reduced NF-κB DNA binding activity and upregulated transcription of the gene for the proteasome C3 subunit. The results in that study were interpreted as indicating that NF-κB is a suppressor of the C3 gene and that the reduced NF-κB DNA binding activity seen in dexamethasone treated myotubes results in increased expression and activity of the C3 gene, ultimately leading to stimulated protein breakdown. It is likely that other genes as well are important for the regulation of protein breakdown in cachectic muscle and that some of those genes may be regulated by the transcription factors discussed here (and probably by other transcription factors as well). In a recent analysis (using the MatInspector V 2.2 program; Quandt et al., 1995) of the promoter of multiple genes in the ubiquitin-proteasome and calpain proteolytic pathways we found potential binding sites for AP-1, NF-κB, and C/EBP in the promoters of ubiquitin, $E2_{14k}$, E3 α, several of the proteasome subunits and calpains, including the muscle specific calpain p94. Further experiments are needed to determine if these genes are indeed regulated by the transcription factors described here and, more importantly, if these genes are involved in the regulation of protein degradation in cachectic muscle.

Outlook

Although most existing data support a role of the ubiquitin-proteasome proteolytic pathway and calpain-dependent release of myofilaments in muscle cachexia during stress, it is likely that other mechanisms are involved as well. For example, recent studies suggest that the ubiquitination of proteins and processing of ubiquitinated proteins by the 26S proteasome may be influenced by the recently described COP9 signalosome (Wei and Deng, 1999). An intriguing observation is that the 8 subunits of the signalosome are identical to the lid components of the 19S capping protein (Schwechheimer and Deng, 2000). Also of interest is the fact that the signalosome subunit CSN5/JAB1 is an important coactivating factor for the c-jun subunit of the AP-1 transcription factor (Chamovitz and Segal, 2001). The potential role of the COP9 signalosome in muscle cachexia is an exiting area for future research.

The proteasome degrades ubiquitinated proteins into peptides and further proteolytic activity "beyond the proteasome" is needed for complete proteolysis into free amino acids. The enzyme tripeptidyl peptidase II (TPP II) may be an important component of the degradation of peptides generated by proteolysis in the proteasome. Although TPP II was discovered almost 20 years ago (Bålöw et al., 1983), it is only recently that its potential relationship with the proteasome has been suggested. Like the COP9 signalosome and the 26S proteasome, TPP II is a "giant" complex consisting of at least eight subunits, each with a molecular weight of 130 kDa (Tomkinson, 1999; Yao and Cohen, 1999). There is evidence that the complete assembly of this complex is needed for its full proteolytic capacity but the mechanisms regulating the assembly of TPP II are poorly understood at present. TPP II cleaves peptides generated by the 26S proteasome into tripeptides and has therefore been characterized as an enzyme that "can count to three" (Tomkinson, 1999). In recent experiments in our laboratory, we have found evidence that TPP II may be involved in the degradation of peptides in muscle from septic rats (C.W. Wray, B. Tomkinson, P.O. Hasselgren, unpublished observations). It will be important in future experiments to determine the role of TPP II (and other peptidases) for the complete hydrolysis of proteins in cachectic muscle.

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