Effects of Serum and Insulin-Like Growth Factor I on Protein Degradation and Protease Gene Expression in Rat L8 Myotubes

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ABSTRACT: We examined the effects of horse and fetal bovine sera and insulin-like growth factor I (IGF-I) on proteolysis and protease gene expression in rat L8 skeletal myotube cultures. Protein degradation was measured as release of radioactive trichloroacetic acid (TCA)-soluble materials from intracellular proteins prelabeled with [3H]tyrosine. Horse serum and fetal bovine serum inhibited (P < .05) protein degradation by 19.7 and 8.1%, respectively. The IGF-I at 200 ng/mL inhibited protein degradation by 14% (P < .01) over a 6-h measurement period. To study the regulation of proteolysis by IGF-I, we evaluated its effects on protease mRNA and α-tubulin mRNA concentrations by Northern blot analysis. Proteases under investigation included cathepsins B and D, proteasome C2 subunit, and m-calpain. The IGF-I had no effect (P > .05) on cathepsin B and D gene expression but slightly increased (P < .05) m-calpain and α-tubulin mRNA concentrations. Proteasome mRNA concentration was reduced (P < .05) by IGF-I treatment. The changes in proteasome mRNA levels paralleled the IGF-I-dependent alterations in proteolysis. These observations suggest that effects of IGF-I on muscle protein degradation may be mediated by the specific down-regulation of proteasomal subunit mRNAs.

Key Words: Insulin-Like Growth Factor, Protein Degradation, Muscles, Proteinases, Gene Expression

Introduction

Insulin-like growth factor I (IGF-I), also called somatomedin C, is a peptide hormone (Froesch et al., 1985; Baxter, 1988). The IGF-I, synthesized and secreted mainly from liver, mediates the growth-promoting actions of growth hormone. The IGF-I shows pleiotropic anabolic effects on skeletal muscle cells, as it does on many other types of cells (Florini, 1987). These include stimulation of amino acid uptake, protein synthesis, glucose uptake, DNA synthesis, RNA synthesis, cell proliferation, and cell differentiation. It is also well-established that IGF-I inhibits protein degradation in muscle (Harper et al., 1987; Roeder et al., 1988; Asakawa et al., 1992; Tomas et al., 1992). Although the effects of IGF-I on muscle protein turnover have been extensively characterized (Kettelhut et al., 1988; Tomas et al., 1991), the molecular and cellular mechanisms by which IGF-I inhibits muscle protein degradation remain unknown.

Like other tissues, skeletal muscle contains multiple proteolytic systems, each of which could play important roles in IGF-I-dependent muscle growth control. Lysosomal proteases, such as cathepsins B, D, H, and L, are present in muscle (Kominami et al., 1984; Goldspink and Lewis, 1985). Muscle contains calcium-dependent proteases, μ-calpain, and m-calpain, which play important roles in myofibrillar protein degradation (Goll et al., 1989, 1992) and a third calpain (p94; Sorimachi et al., 1989) with an unknown function. Muscle also contains proteasome (multicatalytic protease; Fagan et al., 1987), which can degrade proteins in an ATP-dependent manner. Individual roles of the proteinases, their preferred substrates, and endocrine control of their activities have not been fully studied in living cells.

In the present study, we examined the effects of serum and IGF-I on protein degradation and effects of IGF-I on gene expression of major proteolytic systems (cathepsin B, cathepsin D, proteasome, and m-calpain) and α-tubulin of skeletal muscle cells using...
L8 myotube culture. Our hypothesis was that IGF-I would reduce protein degradation and that this would be associated with specific reductions in protease gene expression.

**Experimental Procedures**

**Materials**

L-[ring-3,5-3H]tyrosine (40 to 60 Ci/mmol), [α-32P]dCTP (6,000 Ci/mmol), and Solvable tissue solubilizer were purchased from New England Nuclear (Boston, MA). Fetal bovine (FBS) and horse sera (HS) were from Hyclone (Logan, UT). Dulbecco’s modified Eagle’s medium (DMEM), Hank’s balanced salt solution (HBSS), penicillin/streptomycin solution, and trypsin solution were from Gibco (Grand Island, NY). Trypsin solution was from Gibco (Grand Island, NY). Bio-Safe II scintillation cocktail was from Research Products International (Mount Prospect, IL). Cytosine-β-D-arabinofuranoside (Ara-C), dextran sulfate, denatured salmon testes DNA, ethidium bromide, and bovine serum albumin (BSA) were from Sigma Chemical (St. Louis, MO). Culture dishes and plates were from Corning (Corning, New York). S & S Nytran+ (0.45 μm) membrane was from Schleicher & Schuell (Keene, NH). Denhardt’s solution (100X) was from 5 Prime-3 Prime (Boulder, CO). QIAEX agarose gel extraction kit was from Qiagen (Chatsworth, CA). The random-primer DNA labeling kit was from USB (Cleveland, OH). Formamide, formaldehyde, and trichloroacetic acid (TCA) were from Mallinckrodt (Paris, KY). Quick-Spin G-50 Sephadex columns, human recombinant IGF-I, DIG oligonucleotide tailing kit, and DIG luminescent detection kit were from Boehringer Mannheim (Indianapolis, IN). The 28S oligonucleotides were synthesized by the University of Toronto Hospital for Sick Children Biotechnology Service Center.

**Cell Culture**

The L8 rat skeletal muscle cells were obtained from American Type Culture Collection (Rockville, MD). Cells, stored in liquid nitrogen, were thawed and maintained by repeated subculturing at low density on 10-cm culture dishes. Cells were grown in DMEM supplemented with 10% FBS, 100 units of penicillin/mL, 100 μg of streptomycin/mL, 44 mM NaHCO3, 110 μg of sodium pyruvate/mL in humidified atmosphere of 5% CO2 and 95% air at 37°C. The cells were removed with 25% trypsin in Ca2+- and Mg2+-free HBSS and transferred to 12-well culture plates before experiments. Cells (2.5 × 10⁴ cells/cm²) were grown in the presence of 10% FBS until they reached confluency. At this time the medium was replaced with DMEM containing 2% FBS for induction of differentiation. Approximately 3 d later, when myotube formation was observed, the cells were treated with 10 μM cytosine arabinoside for an additional 48 h to remove any remaining dividing myoblasts. Microscopic examination was used to monitor differentiation. In all studies, fusion percentage was 90 to 95%.

**Measurement of Protein Degradation**

Rates of protein degradation were determined by the release of TCA-soluble radioactivity into the medium at various incubation times after labeling proteins with [3H]tyrosine. Methods were similar to those reported previously (Gulve and Dice, 1989). After complete differentiation, cells were labeled with 1.0 μCi of [3H]tyrosine/mL for 2 d in DMEM containing 10% FBS. Cells were washed once with HBSS and then placed in DMEM containing 10% FBS and 2 mM non-radioactive tyrosine for 2 h at 37°C to allow degradation of short-lived proteins. The cells were then rinsed twice with HBSS and transferred to non-radioactive experimental media containing 2 mM tyrosine. The purpose of adding a high concentration of Tyr was to minimize recycling of released [3H]Tyr and possible underestimation of protein degradation. At the end of the experiment, culture medium was transferred to a microcentrifuge tube containing 100 μL of BSA (10 mg/mL). The TCA was added to a final concentration of 10% (wt/vol). After incubation at 4°C for at least 1 h, samples were centrifuged for 5 min. The precipitates were then dissolved with tissue solubilizer. Cell monolayers were washed with ice-cold phosphate buffered saline (PBS) and solubilized with .5 M NaOH containing .1% Triton X-100. Radioactivities in the cell monolayer and TCA soluble (medium amino acids) and insoluble (medium protein) fractions were measured using a Beckman LS6000 SE scintillation counter. Protein degradation was expressed as the percentage protein degraded over either a 6-h or a 24-h period and was equal to 100 times the radioactivity in the medium amino acids divided by the radioactivity in the medium amino acids plus medium protein plus cell protein.

**Extraction of Total RNA**

Extraction of total RNA has been described previously (Chomczynski and Sacchi, 1987). Briefly, myotube cultures were washed three times with ice-cold PBS and lysed directly on the dishes using 2 mL of Solution A (4 M guanidinium isothiocyanate, 25 mM sodium citrate, pH 7.0, .5% sarcosyl, and 10 mM β-mercaptoethanol). The monolayer was scraped with a rubber policeman to ensure that all cells were released from the dishes. The lysate was transferred to a 15-mL polypropylene tube. To this, 2 mL of 2 M sodium acetate (pH 4.0), 2 mL of phenol, and .4 mL of a chloroform:isoamyl alcohol mixture (49:1) were added sequentially. The lysate was mixed by inversion after each addition. After brief vortexing, the mixture was kept on ice for 15 min and was separated by
centrifugation (12,000 × g) for 30 min at 4°C. The upper aqueous phase was transferred to a new tube and the same volume of ice-cold isopropanol was added. The samples were mixed and stored at −20°C overnight. The RNA was collected by centrifugation (12,000 × g, 20 min at 4°C). The supernatant was discarded and the RNA pellet was resuspended in .4 mL of solution A. One volume of isopropanol was added, and the mixture was stored at −20°C overnight. The RNA pellet was recovered by centrifugation at 12,000 × g for 15 min at 4°C, washed twice with 70% ethanol, and dried under vacuum. The dried RNA pellet was then dissolved in diethylpyrocarbonate-treated water and was quantified by spectrophotometry at a wavelength of 260 nm.

cDNA Probes

The pC2-α, which encodes rat liver proteasome (30.8-kDa C2 subunit; Fujiwara et al., 1989), was provided by Keiji Tanaka (Institute for Enzyme Research, University of Tokushima, Japan). The pHCDpDEco1.1, which encodes human cathepsin D, was provided by John Chirgwin (University of Texas Health Science Center; Faust et al., 1985). The pCB5, which encodes rat liver cathepsin B, was provided by S. J. Chan (Howard Hughes Medical Institute, University of Chicago; Segundo et al., 1985). The pT1, which encodes chicken α-tubulin, was provided by Donald W. Cleveland (Johns Hopkins University; Valenzuela et al., 1981). Rat m-calpain cDNA probe (.9 kbp), which was subcloned in pUC19, was provided by John S. Elce (Queen’s University, Canada; Deluca et al., 1993). Plasmids were amplified and recovered using standard techniques (Maniatis et al., 1982). The cDNA probes encoding proteasome C2 subunit (1,000-bp HindIII/PvuII fragment of pC2-α), cathepsin D (1,000-bp EcorI fragment of pHCDpDEco1.1), cathepsin B (600-bp HindIII fragment of pCB5), α-tubulin (1,500-bp HindIII fragment of pT1), and m-calpain (900-bp EcorI fragment) were prepared as previously described (Ou and Forsberg, 1991; Ilian and Forsberg, 1992). After restriction enzyme digestion, cDNA fragments were separated by electrophoresis and were recovered by electrophoresis onto a dialysis membrane or by using a QIAEX agarose gel extraction kit. The cDNA fragments (25 ng) were labeled with [α-32P] deoxyctytosine triphosphate (dCTP, 6,000 Ci/mmol) using a random-primer kit. Labeled cDNA probes were purified using Quick Spin G-50 Sephadex columns.

Northern Blot Hybridization

The RNA samples (15 µg) were denatured at 65°C for 15 min, applied to a 1.2% agarose gel containing 2.2 M formaldehyde, then electrophoresed at 30 V for 12 h. The quality and the relative abundance of RNA/lane were judged by comparing the ethidium bromide staining of the ribosomal bands. RNA was transferred overnight to Nytran+ nylon membrane and immobilized by baking at 80°C for 40 min. Membranes were incubated at 43°C for overnight in prehybridization buffer (5× SSPE, .2% SDS, 5× Denhardt’s solution, 100 µg/mL of sonicated salmon testes DNA, and 50% formamide). Following prehybridization, the membranes were hybridized for 36 h at 43°C in prehybridization buffer containing 10% dextran sulfate and [32P]cDNA (1,000,000 cpm/mL). After hybridization, membranes were washed three times with 1× SSPE and .1% SDS for 15 min at room temperature and were washed two times with .1× SSPE and .1% SDS for 15 min at 50°C. Membranes were exposed to Kodak X-Omat or Fuji RX film with intensifying screens for 6 to 12 h at −80°C. Quantification of exposures on autoradiographic films was performed using a Bio-Rad model 1650 scanning densitometer and a Hoefer GS-350H scanning program. To remove probe from blots for rehybridization, membranes were washed in 6× SSPE containing 50% formamide at 65°C for 30 min and then rinsed in 2× SSPE. After removal, the membranes were prehybridized and hybridized as indicated above. For normalization, stripped membranes were rehybridized with 28S oligonucleotide probes (5′-AACGATCAGAGTAGTGG-TATTTCACC-3′; Barbu and Doutry, 1989). One hundred nanograms of the 28S oligonucleotides was labeled with digoxigenin-11-UTP by terminal transferase. For hybridization, the probe was diluted with unlabeled oligonucleotide to achieve 10 times the amount of target sequence present in the membrane. Hybridization was performed overnight at 42°C in 5× saline sodium citrate (SSC), .1% N-lauroylsarcosine, .02% SDS, and 1% blocking agent. After hybridization, membranes were washed two times with 1× SSC and .1% SDS for 15 min at room temperature and were washed two times with .1× SSC and .1% SDS for 15 min at 37°C. Membrane-bound probes were detected by DIG-Luminescent kit. Details of methods used for individual studies with sera and IGF-I are presented in the text and in figure legends.

Statistical Analysis

Values are presented as means ± SE. Mean values were compared by Student’s t-test or analysis of variance followed by Fisher’s least-significant difference method for comparing groups (Steel and Torrie, 1980).

Results

Initially, we evaluated the responsiveness of cultured cells to sera to determine whether they would provide an adequate model for the study of proteolysis in vitro. For this purpose, we evaluated the effects of both horse and fetal calf sera on release of radioactivity from pre-labeled myotubes. Recycling of released
Figure 1. Effects of horse serum on protein degradation in cultured L8 myotubes. Myotubes were labeled with 1 μCi/mL of [3H]tyrosine for 2 d, rinsed, and chased for 2 h in Dulbecco’s modified Eagle’s medium (DMEM) + 2 mM tyrosine + 10% FBS as described in Materials and Methods. Cells were rinsed and incubated for 24 h in degradation medium. Degradation medium consisted of DMEM + 2 mM tyrosine supplemented with or without 10% horse serum (HS). Protein degradation is expressed as the percentage (mean ± SE) of labeled protein broken down in 24 h. The study was repeated three times. Error bar indicates ± SE.

[3H]Tyr was minimized by provision of 2 mM Tyr in degradation media. Supplementation of DMEM with horse serum reduced protein degradation by 19.7% during a 24-h measurement period (P < .01; Figure 1). The duration in which horse sera exerted this effect was evaluated in the next study. Figure 2 shows that horse serum continuously repressed proteolysis during a 96-h incubation period.

In the next study we evaluated effects of FBS on protein degradation during a 24-h incubation period (Figure 3). Fetal bovine serum (10% vol/vol) also inhibited protein degradation (P < .05); however, its effects were smaller (8.1% reduction) than those exerted by horse serum.

A difference between adult and fetal sera sources is IGF-I concentration. The IGF-I content of adult sera is higher than that of fetal sera. Insulin-like growth factor I is a major anabolic constituent of sera and is a well-known regulator of protein degradation. Hence, objectives of our next study were to evaluate whether IGF-I could mimic actions of sera on protein degradation in L8 myotube cultures. Insulin-like growth factor I was added to cultures at 200 ng/mL. This level was selected because it is a normal level of IGF-I in rat serum (Asakawa et al., 1992). Others have also used similar levels of IGF-I in related studies (Roeder et al., 1988; Gulve and Dice, 1989). Effects of exposing myotubes to IGF-I for 6 or 24 h on protein degradation are shown in Figures 4A (6 h) and 4B (24 h). Insulin-like growth factor I reduced (P < .001) proteolysis by 14.0 and 26.4% over 6 and 24 h of exposure to IGF-I, respectively.

We postulated that IGF-I-dependent changes in proteolysis may be mediated by changes in proteinase gene expression. Accordingly, we investigated actions of IGF-I on mRNA concentrations encoding several proteinases (cathepsins B and D, proteasome C2 subunit, and m-calpain) using Northern blot analysis. Effects of IGF-I on cathepsin B and D mRNA concentrations are shown in Figures 5 and 6. Scanning densitometry showing effects of IGF-I on cathepsin B and D mRNA concentrations and on mRNAs encoding other proteases and α-tubulin are also shown in Figure 7. Data in Figure 7 have been normalized by expressing proteinase mRNAs as a proportion of 28S rRNA.

The IGF-I treatment had no effect (P > .05) on cathepsin B and D mRNAs (Figures 5 and 6) expressed as a proportion of 28S rRNA (Figure 7). Conversely, IGF-I, after 24 h of exposure, increased (P < .05) m-calpain mRNA by 35% (Figures 7 and 8).

Effects of IGF-I on L8 myotube proteasome C2 subunit mRNA concentration are shown in Figure 9.
Figure 3. Effects of fetal bovine serum on protein degradation in cultured L8 myotubes. Myotubes were labeled with 1 μCi/mL of [3H]tyrosine for 2 d, rinsed, and chased for 2 h in Dulbecco’s modified Eagle’s medium in (DMEM) + 2 mM tyrosine + 10% FBS as described in Materials and Methods. Cells were rinsed and incubated for 24 h in degradation medium. Degradation medium consisted of DMEM + 2 mM tyrosine supplemented with or without 10% fetal bovine serum (FBS). Protein degradation is expressed as the percentage (mean ± SE) of labeled protein broken down in 24 h. The study was repeated six times. Error bar indicates ± SE.

(top panel). Proteasome C2 mRNA was gradually reduced by exposure to IGF-I; an 18% reduction in its concentration was noted at 6 h (Figures 7 and 9) and a 41% reduction was noted at 24 h.

To determine whether changes in protease gene expression mimicked changes in house-keeping gene expression or were the result of more direct regulation by IGF-I, we investigated effects of IGF-I on α-tubulin mRNA concentrations. This was accomplished by stripping the membrane previously exposed to proteasome C2 cDNA and then rehybridizing the membrane to labeled α-tubulin cDNA. Insulin-like growth factor I caused a gradual increase \( (P < .05) \) in α-tubulin mRNA concentration (Figure 7 and Figure 9). Hence, m-calpain behaved as a house-keeping gene in this study, but proteasome C2 subunit mRNA responded in the opposite manner (Figure 7).

Discussion

Horse and fetal bovine sera inhibited proteolysis of L8 myotubes. The inhibition by horse serum was maintained over a 96-h incubation period. This indicates that L8 myotubes are stable over at least 4 d in culture in these experimental conditions and are responsive to the endocrine milieu.

The myofibrillar protein content of cultured myotubes is very low (Gulve et al., 1991) and we therefore expect that the release of radioactivity from cultured cells originated primarily from the non-myofibrillar protein pool. Hence, this study indicates that sera reduced the degradation of non-myofibrillar proteins in cultured myotubes. Its effects on the myofibrillar compartment are not known. It is important to recognize that effects of serum on myofibrillar protein degradation could differ because myofibrillar and non-myofibrillar protein degradation are independently regulated (Goodman, 1987).
Horse serum was more effective in inhibiting proteolysis than FBS. An explanation for this difference could be a difference in the content of IGF-I, which is an inhibitor of proteolysis, in horse vs FBS. Others have reported a higher concentration of IGF-I in adult serum than in fetal serum (Hall and Sara, 1983; Baxter, 1988). In this study, proteolysis in L8 myotubes was effectively inhibited by IGF-I. These results are comparable to the findings of others in various mammalian cells, such as porcine (Hembree et al., 1991), ovine (Harper et al., 1987; Roe et al., 1989), and rat L6 (Roeder et al., 1988) muscle cells and indicate that the ability of serum to attenuate proteolysis in cultured muscle cells may be due to its IGF-I content. Although it is well-known that IGF-I reduces protein degradation in muscle (Gulve and Dice, 1989; Hembree et al., 1991; this study), the molecular mechanisms are not known. To investigate whether regulation of protease gene expression by IGF-I is involved in this process, we examined effects of IGF-I on steady-state mRNA concentrations encoding major proteolytic enzymes using Northern blot analysis. We studied several proteinases that have been identified in skeletal muscle. These included cathepsins B and D (lysosomal cysteine and aspartic proteases, respectively), m-calpain (cytosolic Ca++-activated neutral cysteine protease; CANP) and proteasome (cytosolic and nuclear ATP-dependent protease). We studied the effects of IGF-I on α-tubulin mRNA concentration to determine whether IGF-I-dependent changes in protease gene expression mimic changes in house-keeping gene expression.

Lysosomal proteases degrade sarcoplasmic proteins and may degrade released myofibrillar proteins (Lowell et al., 1986; for review see Goll et al., 1989). Although the roles of cathepsin B and D in muscle protein degradation are not certain, close correlations between cathepsin B and D activities and muscle total protein degradation have been observed (Goldspink and Lewis, 1985; Gerard et al., 1988; Furuno et al., 1990). Insulin-like growth factor I reduced L8 myotube protein degradation but had no effect on cathepsin B or D mRNA concentrations. Hence, IGF-I-dependent changes in myofibrillar protein degradation
are not mediated by changes in cathepsin B or D gene expression.

Calpains have been implicated in degradation of the myofibrillar elements of muscle (Goll et al., 1989, 1992). In addition to this, calpains degrade several other proteins, including protein kinase C (Suzuki et al., 1987), cytoskeletal proteins (Pagi and Lasek, 1984; Billger et al., 1988), and receptors (Poland and Glover, 1988; for review see Goll et al., 1989). Three calpain isoforms (μ-calpain, m-calpain, and p94) with different Ca2+ requirements exist in muscle cells. In this study, we examined effects of IGF-I on gene expression of m-calpain, which requires millimolar concentrations of Ca2+ for activity and which, in muscle, is 1.5 to 3 times more abundant than μ-calpain (Kawashima et al., 1988; Ou and Forsberg, 1991; Ou et al., 1991). Steady-state m-calpain mRNA concentration was increased by IGF-I treatment in a manner that resembled effects of IGF-I on α-tubulin mRNA concentration. This suggests that the changes in calpain gene expression resulted from changes in house-keeping gene expression and that changes in m-calpain gene expression are not involved in the IGF-I-dependent control of proteolysis in muscle cells. In support of m-calpain behaving as a house-keeping gene, house-keeping regulatory elements have been identified in the human m-calpain promoter region (Hata et al., 1989).

Proteasome, a ubiquitous, high-molecular-weight, multicatalytic proteinase, is a major proteolytic system in muscle (Fagan et al., 1987). It degrades proteins in an ATP-dependent manner with or without ubiquitin (Murakami et al., 1992). The core of the proteasome contains 13 to 15 subunits, of which the C2 subunit is the largest (Tanaka et al., 1992). The proteasome may also form the core of the larger 1,500-kDa ATP-dependent protease (Orino et al., 1991). Although proteasome has been implicated in control of myofibrillar protein degradation (Furuno et al., 1990), this role is uncertain (Goll et al., 1989). Proteasome plays important roles in degrading cell cycle-related factors including cyclins, ornithine decarboxylase (Murakami et al., 1992), and several oncogenes. Recent studies have documented an important role for proteasome in MHC Class I-restricted antigen processing (Michalek et al., 1993). In this figure, proteasome was quantified by scanning densitometer. Individual data from each lane were normalized for RNA loading by dividing intensity of exposure with 28S RNA. Values for each point are means of three observations and are expressed as a percentage of control.

Figure 7. Time course showing the effects of IGF-I on the concentration of cathepsin B, cathepsin D, m-calpain, proteasome C2, and α-tubulin mRNAs in L8 myotubes. Autoradiograms shown in Figures 5, 6, 8, and 9 were quantified by scanning densitometer. Individual data from each lane were normalized for RNA loading by dividing intensity of exposure with 28S RNA. Values for each point are means of three observations and are expressed as a percentage of control.
study proteasome C2 mRNA concentration was reduced by IGF-I treatment. This effect was in a direction opposite to the effect of IGF-I on α-tubulin gene expression. This indicates that gene expression of proteasomal subunits plays a role in the adaptation of muscle to IGF-I exposure, and this effect may underlie IGF-I-dependent changes in muscle proteolysis.

In more recent studies (Ou and Forsberg; unpublished observations), we determined that IGF-I reduced degradation of soluble L8 proteins but did not affect degradation of insoluble (myofibrillar and cytoskeletal) proteins. Hence, the IGF-I-dependent decrease in total protein degradation noted in this study is directed at the sarcoplasmic pool and the changes in proteasomal gene expression indicate that IGF-I-dependent control of sarcoplastic protein turnover is associated with changes in proteasome gene expression.

Limitations of this research are that changes in proteinase mRNA concentrations may not bring about changes in proteinase activities. For example, in previous studies (Ilian and Forsberg, 1992) we noted that large changes in μ- and m-calpain mRNAs did not bring about corresponding changes in calpain activities or concentrations in muscle tissue. Considerable opportunity for translational and post-translational regulation of protease activity has been documented. For example, calpains are regulated by calpastatin (Otsuka and Goll, 1987), autolysis (Suzuki et al., 1987), phospholipids (Saido et al., 1992), and possibly, by an activator (Salamino et al., 1993). Furthermore, much of the regulation of protein degradation is mediated by altering a targeted protein’s susceptibility to degradation (Bachmair et al., 1986; Rechsteiner et al., 1987), such as by ubiquitinylation (Hershko et al., 1984), and such changes, therefore, would not be dependent on direct regulation of proteolytic enzyme activities. Finally, individual functions of proteasome’s subunits and of the three calpains are not known. It is possible that IGF-I influences expression of other protease subunits differently than the C2 and m-calpain subunits studied here.

In previous studies we determined that there is substantial potential for regulation of proteinase gene expression in skeletal muscle in vivo (Ilian and Forsberg, 1992). Specifically, during fasting, muscle cathepsin D in particular, but also μ- and m-calpain mRNA, concentrations increase dramatically. Insulin-like growth factor I did not affect the concentrations of these proteinase mRNAs in vitro. Hence, IGF-I is not the major regulator of protease gene expression that operates in vivo during fasting.

Implications

In conclusion, the present study has shown that protein degradation in rat L8 myotubes was inhibited by physiological concentrations of insulin-like growth factor I (IGF-I) as well as by horse and fetal bovine sera. Changes in proteasome steady-state mRNA levels are similar in direction and magnitude to the changes in proteolysis. Therefore, changes in proteasome gene expression may underlie IGF-I-dependent control of protein degradation in muscle cells. Proteasome is regulated by IGF-I in a manner opposite of IGF-I-dependent regulation of house-keeping gene expression, but m-calpain is regulated by IGF-I as a
house-keeping gene. Future studies are needed to identify the signal transduction systems used by IGF-I in muscle cells to regulate protease gene expression.

Literature Cited


