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Regulation of Components of the Ubiquitin System by Insulin-Like Growth Factor I and Growth Hormone in Skeletal Muscle of Rats Made Catabolic with Dexamethasone

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ABSTRACT

To investigate whether the anabolic effects of insulin-like growth factor I (IGF-I) and GH are mediated through regulation of the ubiquitin (Ub) pathway, we examined the effect of IGF-I (0.35 μg/100 g) and/or GH (0.3 mg/100 g BW) on the expression of Ub and Ub-conjugating (E2) enzyme messenger RNAs (mRNAs) in skeletal muscle of rats made catabolic by treatment with dexamethasone (Dex; 0.5 mg/100 g BW) for 3 days. Dex caused a significant loss of body and gastrocnemius weight (14% and 25%, respectively) concurrent with an increase in the 2.8- and 1.2-kb transcripts of Ub (14.3- and 12-fold increases, respectively), the 1.8- and 1.2-kb transcripts of 14-kDa Ub-conjugating enzyme (E2–14 kDa; 5.6- and 7.7-fold increases, respectively), the 4.4- and 2.4-kb transcripts of Ub-E2G (6.5- and 8.2-fold increases, respectively), and the 2E isoform of the 17-kDa E2 mRNA (3.5-fold increase). Injections of IGF-I in Dex-treated animals ameliorated the body weight loss, and the gastrocnemius tended to be heavier. This improvement was also accompanied by a significant suppression of transcripts for Ub (58% and 66% decreases), E2–14 kDa (58% and 68% decreases), and Ub-E2G (78% decrease), whereas the 2E isoform of the 17-kDa E2 was only modestly affected (20% decrease). GH restored serum IGF-I levels to normal in Dex-treated rats, but had no effect on the body weight loss or on any of the studied components of the Ub pathway. Administration of IGF-I to the Dex/GH-treated animals decreased the activated mRNAs of the Ub pathway in a manner and degree similar to those observed in the Dex/IGF-I group.

In summary, these results provide evidence that IGF-I regulates the expression of mRNAs encoding components of the Ub pathway during catabolism and suggest a possible mechanism for the antiproteolytic actions of IGF-I. On the other hand, GH, which is believed not to affect proteolysis but only protein synthesis, had no effect on any of the mRNAs studied. (*Endocrinology* **140:** 5635–5641, 1999)

IN ADDITION to decreased protein synthesis, catabolism is characterized by accelerated proteolysis. In muscle and other lean tissues, protein is broken down by lysosomal enzymes or mitochondrial proteases, or through the ATP-dependent ubiquitin (Ub) pathway. The Ub pathway is believed to be the principal system for the degradation of myofibrillar proteins during food deprivation (1, 2), glucocorticoid treatment (3, 4), metabolic acidosis (5), sepsis (6), tumor cachexia (7), burns (8), and diabetes mellitus (9).

Treatment of rats with glucocorticoids causes an increase in skeletal muscle messenger RNAs (mRNAs) for Ub, the 14-kDa Ub-conjugating enzyme (E2–14 kDa) and subunits of proteasomes, the structure performing proteolysis (3, 4). Also, glucocorticoids are required for the increase in mRNAs encoding Ub and proteasome subunits in muscles of rats subjected to fasting (10), acidosis (11), or sepsis (12).

Injection of GH or insulin-like growth factor I (IGF-I) produces conservation of nitrogen in calorie-restricted (13–15) or glucocorticoid-treated (16, 17) humans and in fasted (18) or dexamethasone (Dex)-treated rats (19, 20). This effect by IGF-I is believed to be accomplished in part by attenuation of proteolysis (18–21), whereas GH appears not to affect protein breakdown (16). To determine whether IGF-I and GH exert these effects by acting on the Ub pathway, we assessed the effects of these peptides on the expression of the mRNAs for Ub and E2 enzymes in skeletal muscle of rats treated with Dex.

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Materials and Methods

Reagents

PCR reagents, DNA polymerase, digoxenin- and biotin-labeled nucleotides, digoxenin chemiluminescent detection reagents, and positively charged nylon membranes were purchased from Roche Molecular Biochemicals (Indianapolis, IN). Reverse transcriptase RNA polymerases, RQ1 ribonuclease (RNase)-free deoxyribonuclease, and restriction enzymes were obtained from Promega Corp. (Madison, WI). Reagents for detection of biotin and for RNase protection assays (RPAs) were obtained from Ambion, Inc. (Austin, TX). Recombinant human GH and IGF-I were gifts from Genentech, Inc. (South San Francisco, CA). Dex sodium phosphate was purchased from American Regent Laboratories Inc. (Shirley, NY), and the purification system for PCR products was obtained from QIAGEN (Chatsworth, CA).

Animals and experimental design

Six-week-old male Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA), weighing 155–165 g, were housed in our animal care facility in 12-h light, 12-h dark cycles and were fed *ad libitum*. At the end of the treatment periods, animals were killed under ether anesthesia, serum was collected, and gastrocnemius muscles were excised, weighed, flash-frozen in liquid nitrogen, and stored at −80 C. The experiments were approved by the institutional animal care and use committee of the University of North Carolina School of Medicine (Chapel Hill, NC).

Exp 1

To determine whether IGF-I decreases the mRNA-encoding components of the Dex-activated Ub pathway, 15 animals were divided ran-

domly into 3 groups. One group received Dex (0.5 mg/100 g BW·day, sc for 3 days), another received Dex and IGF-I (Dex/IGF-I; 0.35 mg IGF-I/100 g BW·day, sc, divided into two daily doses for 3 days), and the third group received only vehicle for 3 days (control group).

Exp 2

To determine whether GH alone or in combination with IGF-I decreases the Dex-activated components of the Ub pathway, 24 animals were divided randomly into 4 groups of 6 animals each. Three groups were treated with Dex, as described above. One of these received only Dex, the second group (Dex/GH) also received GH (0.3 mg/100 g BW·day, sc, for 3 days), and a third group I (Dex/GH/IGF-I) received GH and IGF-I in the doses given above. A fourth group, the controls, received only vehicle.

Probes

The rat cyclophilin complementary DNA (cDNA) clone used in the RPAs was purchased from Ambion, Inc. Rat cDNAs for Ub, E2–14 kDa enzyme, and the 2E isoform of the E2–17 kDa enzyme were prepared by reverse transcribing rat skeletal muscle RNA, then amplifying the cDNAs by PCR using primers designed according to the published sequences (22–24). For Ub, the sense oligo was 5′-GGTAAGACCATCACCCTGGA-3′ [nucleotides (nt) 61–80], and the antisense oligo was 5′-AGGGTGGACTCCTTCTGGAT-3′ (nt 214–233). For E2–14 kDa, the sense oligo was 5′-CAGAAGGGACACCCTTTGAA-3′ (nt 171–190), and the antisense was 5′-AGCTGTGCTGCTTGGCTATT-3′ (nt 410–429). For the 2E isoform of E2–17 kDa, the sense oligo was 5′-CTGCTTTCTATGAGCCCACC-3′ (nt 600–619), and the antisense oligo was 5′-TAGGTTACGCCCGGAGAACT-3′ (nt 758–777). The human Ub-E2G cDNA (25) was amplified from reverse transcribed RNA from GM10 cells. The sense oligo was 5′-CATGAGCCTGGGGAAGATAA-3′ (nt 301–320), and the antisense oligo was 5′-GGTGGGTAGAGTGCAGGAAA-3′ (nt 506–616). The T7 promoter consensus sequence was linked to the 5′-end of each antisense oligo. The amplified PCR products were digested with the appropriate restriction enzymes to confirm their identity. Riboprobes were prepared by *in vitro* transcription and purified with lithium chloride-ethanol precipitation. Riboprobes used for RPAs were purified on 5% acrylamide-8 M urea gel.

Northern hybridization analysis

Total RNA was extracted from gastrocnemius with TRIzol (Life Technologies, Inc., Grand Island, NY). Five or 10 μg RNA were fractionated in 1% agarose gels containing 2.2 M formaldehyde, transferred overnight in 20 × SSC (standard saline citrate) by the capillary method onto positively charged nylon membranes, fixed by UV cross-linking, stained with methylene blue to assure uniform RNA loading, and photographed for normalization of data. Membranes were hybridized overnight at 68 C in a hybridization buffer containing formamide (5 × SSC, 0.1% *N*-laurylsarcosine, 0.02% SDS, 1% blocking reagent, and 50% formamide). Filters were washed twice in 2 × SSC-0.1% SDS at room temperature for 5 min each time, then twice in 0.1 × SSC-0.1% SDS at 68 C for 15 min each time. Chemiluminescent detection of digoxenin-labeled RNA was carried out using the protocol recommended by the manufacturer (26). Blots were exposed repeatedly for different periods of time, and each band was quantified densitometrically using the Image-Pro Plus system (Media Cybernetics, Silver Spring, MD). Each band of interest was normalized to the 18S ribosomal band.

The Ub probe derived from the coding region of the rat polyubiquitin cDNA (see above) hybridized as expected to transcripts of 2.8 and 1.2 kb and after longer exposure to the 0.6-kb transcript, which corresponds to the Ub-ribosomal S30 fusion protein (27). The 2.8- and 1.2-kb transcripts were quantified by densitometry.

To determine whether Dex, GH and IGF-I can affect other classes of E2 enzymes besides the E2–14 kDa, we assessed the expression of Ub-E2G enzyme mRNA, which is believed to be skeletal muscle specific. A human cDNA was used to prepare riboprobes, because the rat sequence is not known. The identity of the amplified Ub-E2G cDNA from the human GM10 cell line was confirmed by restriction mapping and by its hybridization pattern from rat and human RNA from different tissues or cell lines. Ub-E2G transcripts of 4.4, 2.4, and 1.6 kb were easily detected in skeletal muscle, and after long exposure faint signals were observed in some other tissues.

RPA

RPAs were performed as reported previously (28). Briefly, 5 μg total RNA derived from gastrocnemius muscle were hybridized overnight at 45 C with 3 fmol biotin-labeled cyclophilin and E2–14 kDa or with the 2E isoform of the E2–17 kDa antisense RNAs. After hybridization, samples were treated with A and T1 RNases (Ambion, Inc.) at 37 C for 30 min. Protected fragments were separated in 8% acrylamide-8 M urea gels and transferred to positively charged nylon membranes by electroblotting. Pilot experiments using constant amount of probes but different concentrations of RNA confirmed that the concentration of probes was in molar excess. Yeast RNA hybridized with the above probes confirmed that the digestion was complete. For washing and detecting the signal, protocols recommended by the manufacturer (Ambion, Inc.) were followed. The signal of the E2–14 kDa or 2E isoform of E2–17 kDa was normalized to cyclophilin and expressed as the ratio of the two signals.

Measurement of IGF-I in serum

Serum IGF-I concentrations were measured by RIA after removal of IGF-I-binding proteins (IGFBPs) using ODC-silica cartridge chromatography (C_{18} Sep-Pak, Waters Corp., Milford, MA) (29).

Statistics

All values are presented as the mean ± SEM. One-way ANOVA was used to determine the significance of differences among three or more experimental groups. Unpaired *t* test was used for two groups, and the *P* value was calculated with the Newman-Keuls post test. $P < 0.05$ was considered significant.

Results

IGF-I ameliorates the weight loss caused by Dex

In the first experiment, the two groups of animals treated with Dex had significant loss of body weight (Table 1), with the Dex/IGF-I-treated animals loosing less weight than those treated with Dex alone ($P = 0.004$). In the second experiment, GH did not prevent the body weight loss produced by Dex, but addition of IGF-I produced significant attenuation of weight loss (Dex/GH/IGF-I *vs.* Dex, $P < 0.01$; Dex/GH/IGF-I *vs.* Dex/GH, $P < 0.05$; Table 2).

Dex treatment also reduced the weight of the gastrocnemius muscle in both experiments (Tables 1 and 2), and they were made slightly heavier by concurrent treatment with IGF-I, but this difference did not reach statistical significance ($P > 0.05$).

Dex increases the expression of genes encoding components of the Ub pathway

In the first experiment, Dex caused a 14.3-fold increase in the 2.8-kb transcript and a 12-fold increase in the 1.2-kb

TABLE 1. Effect of dexamethasone (Dex) and IGF-I treatment on body weight and weight of gastrocnemius muscle

	Control	Dex	Dex/IGF-I
Initial BW (g)	212 ± 1.5	211.8 ± 2.7	213.2 ± 3.4
Final BW (g)	232.4 ± 5.9	184.7 ± 0.9[a]	195.2 ± 4.3[a,b]
% Decrease in BW		14.6 ± 1.02	9.3 ± 0.88[c]
Gastrocnemius wt (g)	1.45 ± 0.04	1.1 ± 0.02[a]	1.2 ± 0.03[a]

[a] $P < 0.001$ *vs.* control.
[b] $P < 0.05$ *vs.* Dex.
[c] $P < 0.01$ *vs.* Dex.

TABLE 2. Effect of dexamethasone (Dex), GH, and GH/IGF-I treatment on body weight and weight of gastrocnemius muscle

	Control	Dex	Dex/GH	Dex/GH/IGF-I
Initial BW (g)	199.3 ± 2.0	196.5 ± 4.6	200.7 ± 0.85	194.7 ± 3.2
Final BW (g)	224.9 ± 2.7	169.7 ± 3.6[a]	172.9 ± 1.7[a]	174.3 ± 3.2[a]
% Decrease in BW		13.7 ± 0.6	13.9 ± 0.8	11.0 ± 0.2[b]
Gastrocnemius (g)	1.225 ± 0.03	0.918 ± 0.02[c]	0.913 ± 0.02[c]	0.952 ± 0.02[c]

[a] $P < 0.001$ *vs.* control.
[b] $P < 0.05$ *vs.* Dex/GH; $P < 0.01$ *vs.* Dex.
[c] $P < 0.01$ *vs.* control.

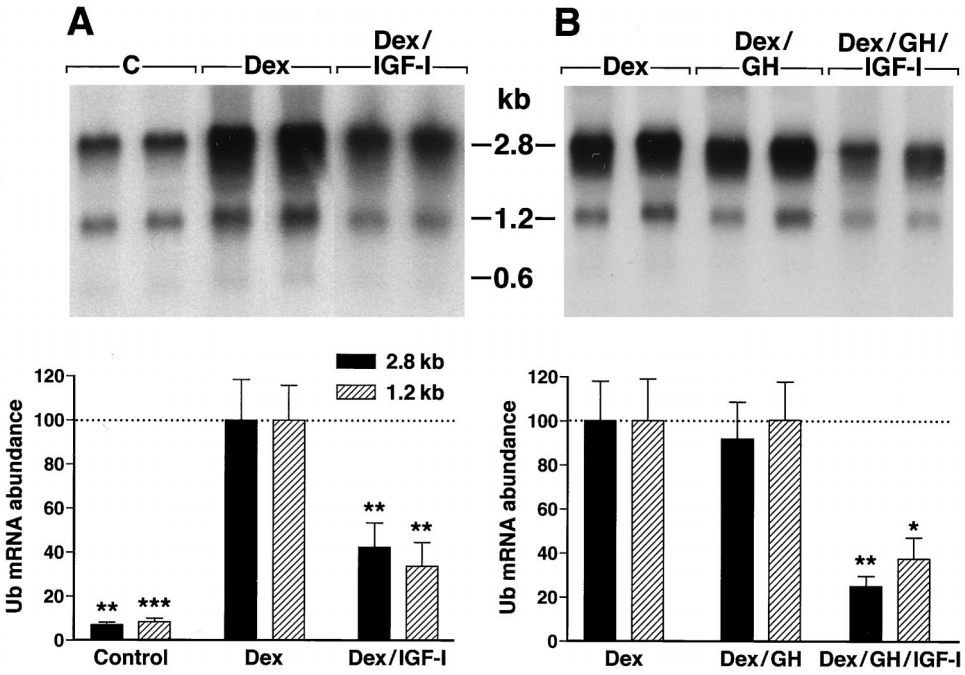

FIG. 1. The effects of Dex, IGF-I, and GH on Ub transcripts. Five micrograms of total RNA from gastrocnemius muscles were hybridized with digoxenin-labeled Ub riboprobe. Two transcripts were identified by Northern hybridization analyses, one at 2.8 kb (*black bars*) and one at 1.2 kb (*hatched bars*). After a prolonged exposure, a faint transcript at 0.6 kb was identified. A, First animal experiment. *Top*, Representative Northern blot. C, Control group; Dex, Dex-treated group; Dex/IGF-I, Dex- and IGF-I-treated group. *Bottom*, Autoradiographic signals of 2.8- and 1.2-kb transcripts were scanned, normalized to the 18S ribosomal band, and expressed as a percentage of the Dex group signal. **, $P < 0.01$; ***, $P < 0.001$ (*vs.* Dex group). B, Second animal experiment. *Top*: Dex, Dex alone; Dex/GH, Dex and GH; Dex/GH/IGF-I, Dex, GH, and IGF-I. *Bottom*, The 2.8- and 1.2-kb transcripts of Ub were scanned, normalized to the 18S ribosomal band, and expressed as the percentage of the Dex group. *, $P < 0.05$; **, $P < 0.01$ (*vs.* Dex and Dex/GH groups).

transcript of Ub compared with control values ($P < 0.01$ and $P < 0.001$, respectively; Fig. 1A). The transcripts for E2–14 kDa enzyme RNA were also up-regulated by Dex; the 1.8-kb transcript was increased 5.6-fold, and the 1.2-kb transcript was increased 7.7-fold ($P < 0.001$ and Fig. 2A). A similar increase was observed for the transcripts encoding the Ub-E2G enzyme. The 4.4-kb transcript was increased 6.5-fold compared with the control value ($P < 0.001$), and the 2.4-kb transcript was increased 8.2-fold ($P < 0.01$; Fig. 3A). Similar results were obtained from the second animal experiment (data not shown).

IGF-I reduces the activated components of the Ub pathway (Exp 1)

Concurrent administration of IGF-I to Dex-treated animals significantly reduced the mRNAs for Ub, E2–14 kDa, and Ub-E2G enzymes. IGF-I decreased the 2.8-kb transcript of Ub by 58% ($P < 0.01$) and the 1.2-kb transcript by 66% ($P < 0.01$; Fig. 1A). The reduction caused by IGF-I in each transcript, however, did not reach the levels in the control animal ($P > 0.05$).

The 1.8-kb transcript of the E2–14 kDa was decreased by 58% ($P < 0.001$), and the 1.2-kb transcript was decreased by 68% ($P < 0.001$; Fig. 2A). These results were confirmed with RPA using the same probe as that used for Northern hybridization analysis. This probe, derived from the coding region, gives a protected fragment common to both the 1.8- and 1.2-kb transcripts. Dex caused a 6-fold increase compared with the controls, and IGF-I produced a 66% decrease from that observed in the animals treated with Dex only (Fig 4).

As with Ub and E2–14 kDa, the 4.4- and 2.4-kb transcripts for Ub-E2G were reduced by IGF-I by 78% (Dex/IGF-I *vs.* Dex, $P < 0.001$ and $P < 0.01$, respectively; Fig. 3A).

The effect of IGF-I on the 2E isoform of the E2–17 kDa mRNA was minimal compared with the effect on other components of the pathway. Dex caused a 3.5-fold increase, but IGF-I decreased this transcript by only 20% ($P > 0.05$; Fig 5).

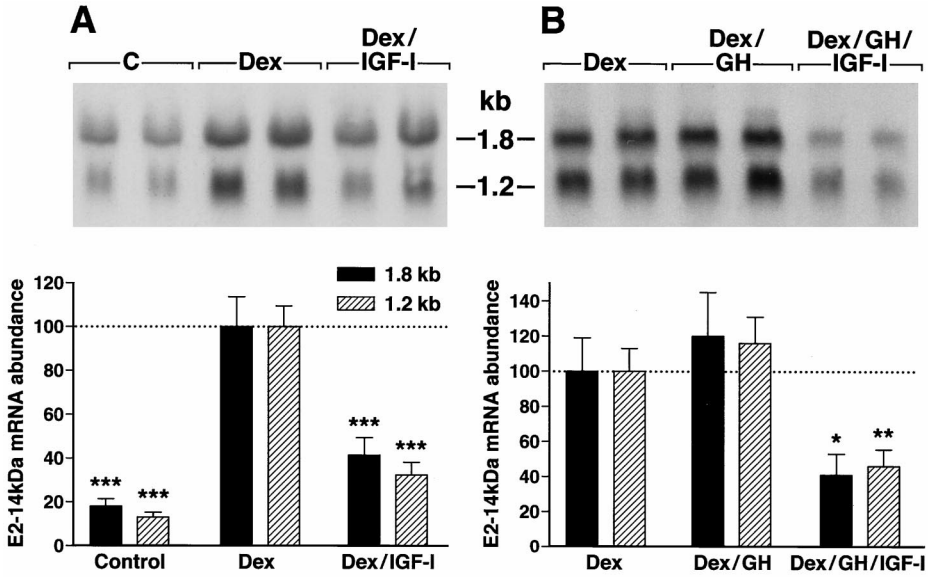

FIG. 2. The effects of Dex, IGF-I, and GH on the E2–14 kDa transcripts. Ten micrograms of total RNA from gastrocnemius muscles were hybridized with the digoxenin-labeled E2–14 kDa riboprobe. Transcripts of 1.8 and 1.2 kb were identified by Northern hybridization analysis. A, Same as in Fig. 1. ***, $P < 0.001$ *vs.* Dex. B, Same as in Fig. 1. *, $P < 0.05$; **, $P < 0.01$ (*vs.* Dex and Dex/GH groups).

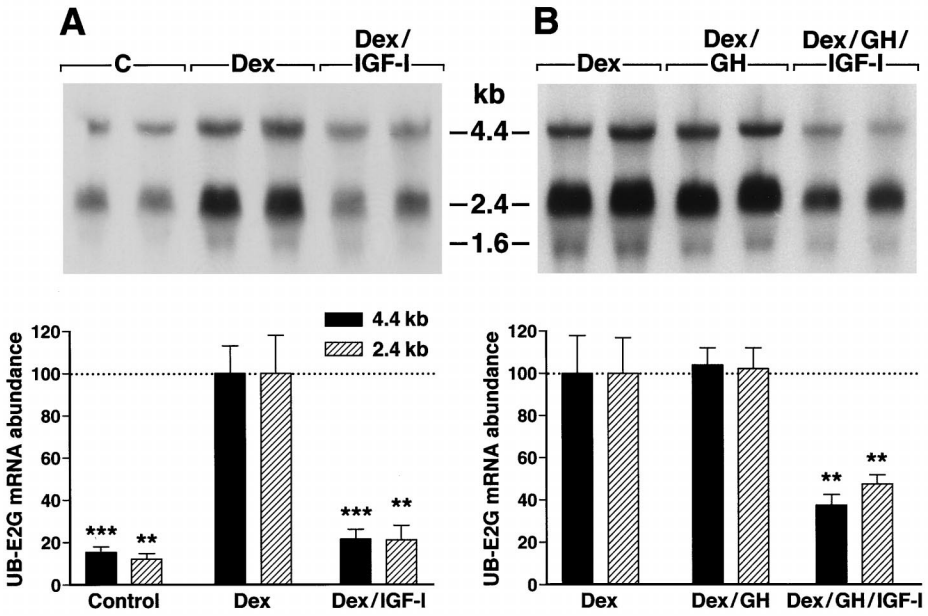

FIG. 3. The effects of Dex, IGF-I, and GH on the Ub-E2G transcripts. Ten micrograms of total RNA from gastrocnemius muscles were hybridized with digoxenin-labeled Ub-E2G riboprobe. Transcripts of 4.4 and 2.4 kb were identified by Northern hybridization analysis along with a faint transcript of 1.6 kb. A, Same as in Fig. 1. **, $P < 0.01$; ***, $P < 0.001$ (*vs.* Dex group). B, Same as in Fig. 1. **, $P < 0.01$ (*vs.* Dex and Dex/GH groups).

GH has no effect on the activated components of the Ub pathway (Exp 2)

In Exp 2, concurrent administration of GH to Dex-treated animals had no effect on the Ub pathway mRNAs, whereas addition of IGF-I treatment caused reductions similar to those observed in the first experiment. Both of the transcripts encoding Ub were quantitatively similar in the Dex and Dex/GH groups ($P > 0.05$), whereas addition of IGF-I caused 74% and 62% decreases ($P < 0.01$ and $P < 0.05$, respectively; Fig. 1B). After injection of IGF-I, the Ub transcripts in the Dex/GH animals were not significant different from those in controls ($P > 0.05$).

The 1.8- and 1.2-kb transcripts of E2–14 kDa also were not unaffected by GH ($P > 0.05$; Dex/GH *vs.* Dex), but IGF-I caused a significant decrease in both transcripts compared with Dex alone (59% and 54%, respectively; $P < 0.05$ and $P < 0.01$, respectively; Fig. 2B).

The 4.4- and 2.4-kb transcripts of Ub-E2G were similar in Dex- and Dex/GH-treated animals ($P > 0.05$, respectively), whereas addition of IGF-I caused 62% and 53% decreases ($P < 0.01$; Fig. 3B).

Serum IGF-I was measured in the second animal experiment to evaluate the efficacy of GH administration. Dex decreased the serum level of IGF-I by 30% compared with that in control animals (1895 ± 75 *vs.* 1324 ± 139 ng/ml; $P < 0.05$), and GH increased IGF-I to the normal level (2205 ± 198 ng/ml; $P < 0.01$ *vs.* Dex).

Discussion

We report that gastrocnemius muscles made catabolic by Dex treatment are reduced in weight and that there is concurrent up-regulation of the mRNAs for Ub and the E2–14 kDa enzymes. We also observed increases in mRNAs encoding two other E2 enzymes, the Ub-E2G and the 2E isoform

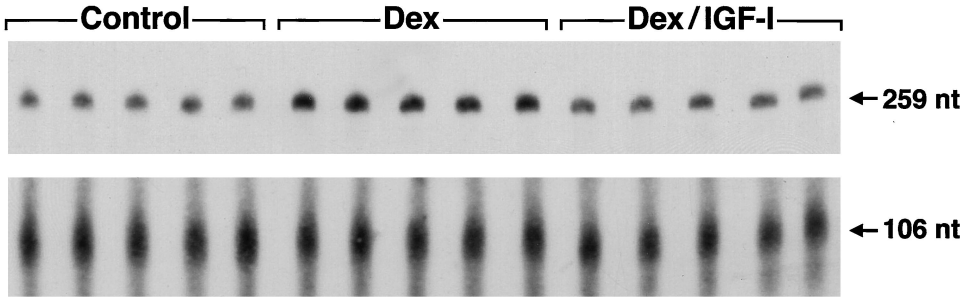

FIG. 4. The effects of Dex and IGF-I on E2–14 kDa mRNA assessed by RPA. Three femtomoles of gel-purified biotin-labeled E2–14 kDa and rat cyclophilin riboprobes were cohybridized with 7 μg total RNA from gastrocnemius muscle (first animal experiment) and produced bands of the expected sizes of 259 and 106 nt, respectively. Dex caused a 6-fold increase in the E2–14 kDa transcript compared with that in control animals (C), whereas animals treated with Dex and IGF-I (Dex/IGF-I) had a 66% decrease compared with animals treated with Dex alone.

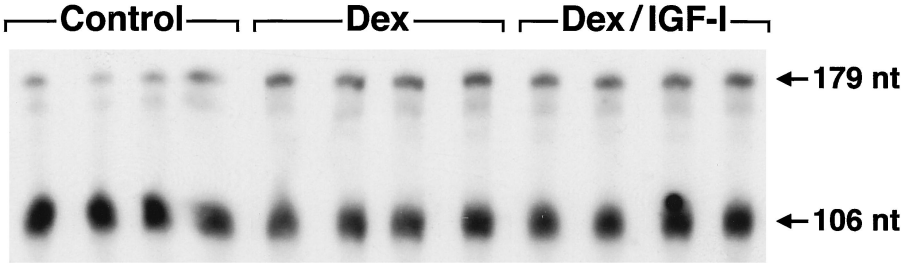

FIG. 5. IGF-I has no significant effect on the 2E isoform of the E2–17 kDa enzyme transcript that is up-regulated by Dex. Ten micrograms of total RNA from gastrocnemius muscle (first animal experiment) were cohybridized with 3 fmol biotin-labeled, gel-purified 2E isoform of E2–17 kDa and rat cyclophilin riboprobes, giving the protected fragments of the expected sizes (179 and 106 nt, respectively). Dex caused a 3.5-fold increase in the 2E isoform compared with that in control animals (C), but the addition of IGF-I (Dex/IGF-I) had a minimal effect on this transcript (20% decrease; $P > 0.05$ *vs.* Dex).

of the 17-kDa enzyme, indicating that multiple E2 enzymes are activated. Coadministration of IGF-I attenuates the effects of Dex on Ub system mRNAs, whereas GH does not. We used Dex to produce catabolism because it is a well characterized model (30–33) that causes myofibrillar degradation and concurrent activation of Ub, E2–14 kDa enzyme, and subunits of proteasome mRNAs (3, 4). Suggesting that up-regulation of Ub is linked to decreased muscle weight is the observation that administration of the glucocorticoid receptor antagonist RU 38486 abolishes the increase in Ub mRNA caused by Dex (34) and attenuates the loss of body and muscle weights (35).

Our study provides evidence that IGF-I opposes the catabolic effect of glucocorticoids by acting on the Ub pathway. Two of the major components of this pathway, Ub and E2 enzymes, are down-regulated by IGF-I. Moreover, this effect appears to be relatively specific, because the 2E isoform of the 17-kDa E2 enzyme was affected only slightly by IGF-I. In burn injury, where proteolysis occurs mainly through activation of the Ub pathway (8), IGF-I decreases proteolysis and the expression of Ub mRNA (36).

IGF-I might act on the Ub system by decreasing the transcription rate and/or stability of mRNAs. Given that E2 enzymes catalyze thermodynamically irreversible and rate-limiting reactions (37), down-regulation of these enzymes could cause a decrease in the expression of Ub by a feedback mechanism. In cultured L6 myotubes, IGF-I makes the E2–14 kDa mRNA more unstable, but has no effect on its transcription rate (38). However, similar data on the stability of mRNAs encoding Ub and other E2 enzymes are lacking. Many mRNAs with decreased stability (39) contain adenylate/uridylate-rich elements in the 3′-untranslated region (3′UTR). Also, mRNAs with increased numbers of AUUUA motifs have been observed to have increased turnover (40). The 3′UTR of the E2–14 kDa gene contains at least three AUUUA motifs and several AU-rich regions, but it is not known whether these are involved in the increased turnover produced by IGF-I. Interestingly, the 3′UTR of the Ub-E2G gene, which is also down-regulated by IGF-I, contains two AUUA repeats in a known sequence of 89 nt, pointing toward less stability. On the other hand, 3′UTR of the 2E isoform, which was minimally affected by IGF-I, has only one AUUUA motif in a known sequence of 370 nt.

We assume that the down-regulation by IGF-I of the mRNAs for Ub and E2 enzymes and the attenuated loss of body weight reflect decreased proteolysis rather than increased protein synthesis alone. This assumption is based, first, on the observation that proteolysis in this model is increased for the first 4 days then returns to normal, whereas protein synthesis remains decreased (3, 30). Secondly, both IGF-I and GH effect anabolism by increasing protein synthesis, but only IGF-I has been reported to attenuate proteolysis (16, 18–21). In our experiments, only IGF-I treatment improved body weight, whereas GH had no effect on weight loss or on Ub pathway mRNAs. Finally, in burn injury, another model of catabolism in which the Ub pathway is activated (8) and glucocorticoids are required (41), administration of IGF-I decreases total and myofibrillar proteolysis

concomitant with a decrease in the expression of the Ub mRNA (36).

The observation that GH has no effect on body weight loss or on expression of the Ub pathway mRNAs suggests that it has no significant effect on proteolysis despite the fact that GH treatment increased serum IGF-I. This could be explained if some GH actions are not mediated through IGF-I or if other GH-dependent factors alter the capacity of IGF-I to regulate these mRNAs. IGFBPs could be such factors, because they are regulated differently by GH and IGF-I, and they modify the actions and bioavailability of IGF-I (42). In cultured L6 myotubes, des(1–3)-IGF-I, which has low affinity for the IGFBPs, is more potent than IGF-I in decreasing the levels of the 14 kDa E2 mRNA (38). Similarly, smaller doses of the IGF-I variants that have reduced affinity for IGFBPs are needed in Dex-treated rats to produce growth-promoting effects comparable to those of IGF-I (19). Skeletal muscles express IGFBP-4, -5, and -6 (43). IGFBP-5 is GH and IGF-I dependent in skeletal muscles (44) and enhances the actions of IGF-I, whereas IGFBP-4 inhibits them (42). It is difficult, however, to explain the differences in the effects of IGF-I and GH on the Ub pathway mRNAs based only on the IGFBPs, because Dex/GH/IGF-I had an effect comparable to Dex/IGF-I. Finally, our administration of GH to the Dex-treated rats may not have produced the serum levels of IGF-I required for suppression of the Ub pathway or may not have produced the required autocrine/paracrine actions of IGF-I. *In vitro* IGF-I concentrations at or above 80 ng/ml have a maximal effect on the degradation of the E2–14 kDa mRNA (38), and in our study, systemic administration of GH produced serum IGF-I values in the normal range.

Our findings provide insight into the mechanisms by which IGF-I exerts its antiproteolytic actions. Given that the Ub pathway is involved not only in protein degradation in catabolic states but also in degradation of transcription factors and cyclins, DNA repair, and protein translocation, this action of IGF-I could provide links to many cellular events.

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