JCI The Journal of Clinical Investigation

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J Clin Invest. 1995;96(5):2113-2119. https://doi.org/10.1172/JCI118264.

Research Article

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Sensitivity and Protein Turnover Response to Glucocorticoids Are Different in Skeletal Muscle from Adult and Old Rats

Lack of Regulation of the Ubiquitin-Proteasome Proteolytic Pathway in Aging

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Abstract

We studied glucocorticoid-induced muscle wasting and subsequent recovery in adult (7-mo-old) and old (22-mo-old) rats, since the increased incidence of various disease states may result in glucocorticoids hypersecretion in aging. Adult and old rats received dexamethasone in their drinking water and were then allowed to recover. Muscle wasting occurred more rapidly in old rats and the recovery of muscle mass was impaired, suggesting that glucocorticoids may be involved in the emergence of muscle atrophy with advancing age. According to measurements in incubated epitrochlearis muscles, dexamethasone-induced muscle wasting mainly resulted from increased protein breakdown in the adult, but from depressed protein synthesis in the aged animal. Increased expression of cathepsin D, m-calpain, and ubiquitin was observed in the muscles from both dexamethasone-treated adult and old rats. By contrast, the disappearance of the stimulatory effect of glucocorticoids on protein breakdown in aging occurred along with a loss of ability of steroids to enhance the expression of the 14-kD ubiquitin carrier protein E2, which is involved in protein substrates ubiquitinylation, and of subunits of the 20 S proteasome (the proteolytic core of the 26 S proteasome that degrades ubiquitin conjugates). Thus, if glucocorticoids play any role in the progressive muscle atrophy seen in aging, this is unlikely to result from an activation of the ubiquitin-proteasome proteolytic pathway. (*J. Clin. Invest.* 1995. 96:2113–2119.)
Keys words: aging • muscle • glucocorticoids • protein turnover • ubiquitin • proteasome

Introduction

Circulating levels of glucocorticoids are elevated in Cushing's syndrome, infections, stresses, and various traumatic conditions or clinical treatments (1–5). Although, the association of glucocorticoid excess and muscle atrophy is well established, the mechanisms underlying muscle wasting are not totally elucidated. An inhibition of skeletal muscle protein synthesis has been consistently observed in glucocorticoid-treated young animals (6–10). The effects of glucocorticoids on skeletal muscle protein breakdown are less clear. Increased proteolysis has been reported in fed animals receiving large doses of steroids (6, 8, 10), but not in all studies (7, 9). By contrast, glucocorticoids are required for the increase in proteolysis seen in fasting (see reference 11 for a review), as well as in some pathologic states (e.g., acidosis [12] or thermal injury [13]). During acidosis, it appears, however, that glucocorticoids may play a secondary role in the perturbations of skeletal muscle protein turnover (14).

Skeletal muscles, like other mammalian tissues, contain several proteolytic systems. The best known proteolytic pathway is the lysosomal process. Muscles also contain two major cytosolic proteolytic pathways, Ca^{2+}-dependent (15) and ATP-ubiquitin–dependent (16), respectively. The major lysosomal proteinases (cathepsins B, H, L and D) and the Ca^{2+}-dependent proteinases (μ- and m-calpains that differ in their affinities for Ca^{2+}), do not contribute significantly to increased skeletal muscle proteolysis in many muscle wasting conditions, and do not play a major role in the degradation of myofibrillar proteins (17–19). By contrast, the ATP-ubiquitin–dependent proteolytic pathway, which was previously widely believed to degrade abnormal and short-lived proteins (20, 21), is presumably the critical system responsible for the breakdown of the long-lived contractile components (18, 19, 22). In this pathway, ubiquitin first covalently binds to protein substrates in a multistep process requiring ATP, and serves as a signal for degradation (21). Ubiquitin-protein conjugates are then preferentially degraded by a very large 1,500-kD (26 S) proteolytic complex that also requires ATP for activation and substrate hydrolysis (21, 23). The 26 S complex consists of the 20 S proteasome core, a multicatalytic proteinase composed of 14 different subunits, plus regulatory components containing several proteins including ATPases (21, 23). Recent experiments have shown that glucocorticoids regulate the ATP-ubiquitin dependent proteolytic pathway in muscles from starved and acidotic rats, apparently by increasing the expression of ubiquitin and of 20 S proteasome subunits (14, 24). To our knowledge, the effects of glucocorticoids on gene expression of lysosomal or Ca^{2+}-dependent proteinases are unknown in skeletal muscle.

During aging, a progressive loss of muscle mass is well described both in humans (25) and rodents (26, 27). This loss of proteins results from an imbalance between protein synthesis and degradation rates. This imbalance, however, is not clearly apparent when basal rates of protein turnover are measured (4, 28–30), but can be detected in rats for example during the postprandial period or endurance training (31). Mechanisms underlying changes in the regulation of muscle protein turnover during aging are still totally obscure. In the present study we hypothetized that glucocorticoids may play a role since there are periods of glucocorticoids hypersecretion with advanced age

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Received for publication 15 May 1995 and accepted in revised form 1 August 1995.

J. Clin. Invest.

0021-9738/95/11/2113/07 $2.00
Volume 96, November 1995, 2113–2119

(32), and increased susceptibility to illness. In addition, the return of circulating corticosterone to basal levels is slowed down in old stressed rats (32). Thus, muscle atrophy during aging may partly result from an inability to rapidly restore muscle protein mass when challenged by a catabolic state. To study how glucocorticoids may affect skeletal muscle protein turnover in adult and aging rats, we examined in the present experiments the changes in muscle protein synthesis and breakdown and in the abundance of mRNA encoding cathepsins D and B, m-calpain, and critical components of the ubiquitin-proteasome proteolytic pathway (i.e., ubiquitin, 14-kD ubiquitin carrier protein E2, and subunits of the 20 S proteasome) following dexamethasone treatment and subsequent recovery.

Methods

Animals. Adult (6–8 months) and old (22 months) male Sprague-Dawley rats were purchased from Iffa-Credo (L'Arbresle, France) and housed under controlled environmental conditions (temperature 22°C; 12-h dark period starting at 1800 h). Rats were given free access to commercial laboratory chow and water before the experiments were performed. Both adult and old rats were randomly divided into a control and a dexamethasone-treated group. Dexamethasone (DEX)[1] (a synthetic glucocorticoid analogue that does not bind to plasma binding proteins) was given daily (at 0900 h) through the drinking water. DEX concentration was adjusted every day on the basis of drinking water intake the day before in order to obtain constant daily doses to all animals (507±185 and 476±45 mg/kg per day in adult and old rats, respectively). These doses were chosen on the basis of preliminary experiments that showed that these doses corresponded to a maximum but reversible effect of DEX on muscle protein wasting. Rats did not recover from DEX treatment when higher doses (i.e., 1500–2000 mg/kg per day) were tested; lower doses resulted in lesser effects. As DEX has been reported to decrease food intake, all groups were pair-fed to the group that had the lowest food intake (i.e., DEX-treated old rats). DEX was given for 5 d in old rats but for 6 d in adults in order to generate a similar muscle atrophy in both groups, since muscle wasting was more rapid in aged animals (see below). Rats were allowed to recover for either 3 (R + 3) or 7 (R + 7) days. Animals were killed under anesthesia with sodium pentobarbital (6.0 mg/100 gram body weight) after an overnight fast. Blood samples were collected for glycemia assessment and for measuring plasma insulin levels. Epitrochlearis muscles were dissected intact for incubation (see below) and gastrocnemius/plantaris complex was frozen in liquid nitrogen and stored at −80°C until analysis.

Rates of protein turnover. Protein turnover was measured as previously described (33). Briefly, epitrochlearis muscles were preincubated for 30 min in Krebs Henseleit Buffer (KHB) (NaCl 120 mM, KCl 4.8 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM and $MgSO_4$ 1.2 mM; pH 7.4), supplemented with 5 mM glucose, 5 mM Hepes, 0.1% BSA, 0.17 mM leucine, 0.20 mM valine, and 0.10 mM isoleucine, and saturated with 95% O2–5% CO2 gas mixture. Muscles were then transferred into fresh media of the same composition containing 0.5 mM L-[^{14}C]phenylalanine (0.15 μCi/ml) for 60 min. At the end of the incubation, muscles were blotted and homogenized in 10% trichloroacetic acid (TCA). TCA-insoluble material was washed three times with 10% TCA, and solubilized in 1 N NaOH at 37°C for determination of protein-bound radioactivity and protein content. Protein synthesis was expressed as nanomoles of phenylalanine incorporated per milligram protein per hour.

Protein degradation was determined as described by Tischler et al. (34). Muscles were incubated as mentioned above. Since tyrosine is neither synthesized nor degraded by muscle, the release of this amino acid from muscle into the incubation medium directly reflects the net protein breakdown. Total protein breakdown (expressed in nanomoles of tyrosine per milligram protein per hour) was calculated as the sum of net protein breakdown and protein synthesis, after converting the rate of phenylalanine incorporation into proteins in tyrosine equivalents (34).

Northern blot analysis. To further identify the proteolytic pathway(s) responsible for increased skeletal muscle proteolysis during the DEX treatment, the expression of the mRNAs encoding proteinases and cofactors involved in lysosomal (cathepsins D and B), Ca^{2+}-dependent (m-calpain) or ATP-ubiquitin-dependent (ubiquitin, 14-kD ubiquitin carrier protein E2 (14-kD E2), and C2 or C9 proteasome subunits) proteolytic pathways was examined. Total RNA was extracted from 0.5 grams of gastrocnemius/plantaris complex by the method of Chomczynsky and Sacchi (35), separated in formaldehyde agarose gels (1%), and transferred electrophoretically to nylon membranes (GeneScreen, Dupont de Nemours des Ulis, France). RNA was covalently bound to the membrane by UV cross-linking.

Membranes were hybridized with cDNA probes encoding chicken polyubiquitin (36), the rat 14-kD E2 (37), the C2 and C9 rat proteasome subunits (38, 39), the human m-calpain (40) and the human cathepsin D (41). Hybridizations were conducted overnight at 65°C with [^{32}P]-cDNA fragments labeled by random priming. After washing at the same temperature, filters were autoradiographied at −80°C with intensifying screens on Hyperfilm-MP (Amersham, Buckinghamshire, England). A cDNA probe encoding rat cathepsin B (42) was subcloned into EcoRI sites of pGem-blue and was linearized with HindIII as previously described (43). Membranes were hybridized at 60°C with a single stranded antisense cathepsin B riboprobe synthetized using T7 RNA polymerase and digoxigenin-labeled UTP (Boehringer Mannheim, Indianapolis, IN). After washings, specific hybridization was revealed autoradiographically using chemiluminescence reaction as described by the manufacturer. Preliminary experiments showed that the intensity of the mRNAs was proportional to the amount of total RNA loaded, and to the duration of exposure of the films. All RNA samples from DEX-treated and pair-fed animals of the same age were run on the same gel.

After stripping of the different probes, filters were reprobed with a cDNA fragment encoding the "housekeeping gene" glyceraldehyde-3-phosphate dehydrogenase (GADPH) (44). Autoradiographic signals were quantified in arbitrary units using a densitometer, and normalized using the corresponding GAPDH signals to correct for variations in RNA loading.

Analytical methods. Tyrosine was determined fluorometrically (45) and protein assayed by the bicinchoninic acid procedure (BCA; Pierce, Rockford, IL). RNA was quantified by UV spectroscopy at 260 nm. Plasma insulin was measured by RIA using human insulin as standard as previously described (46). Blood glucose was assessed enzymatically using glucose oxidase (Boehringer Mannheim).

Statistics. Data are expressed as means±SEM of the absolute or percent difference between values from control and DEX-treated animals of the same age. The significance of differences was analyzed by Student's *t* test.

Results

Animals characteristics. Food intake was maintained at similar levels in all groups during both the DEX treatment (23.9, 12.7, 5.1, 3.2, 2.0, and 2.0 grams at day 1, 2, 3, 4, 5, and 6, respectively) and the recovery period (4.0, 7.8, 15.9, 20.1, 27.1, 30.0, and 30.0 grams at day 1, 2, 3, 4, 5, 6, and 7, respectively). Thus, differences between groups do not originate from different intakes. DEX induced a progressive weight loss of 100–130 grams over the treatment period with animals showing no apparent distress. Epitrochlearis atrophy was similar in adult and old rats at the end of the DEX treatment (−29.3±3.4 and

1. *Abbreviations used in this paper:* DEX, dexamethasone; 14-kD E2, 14-kD ubiquitin carrier protein E2.

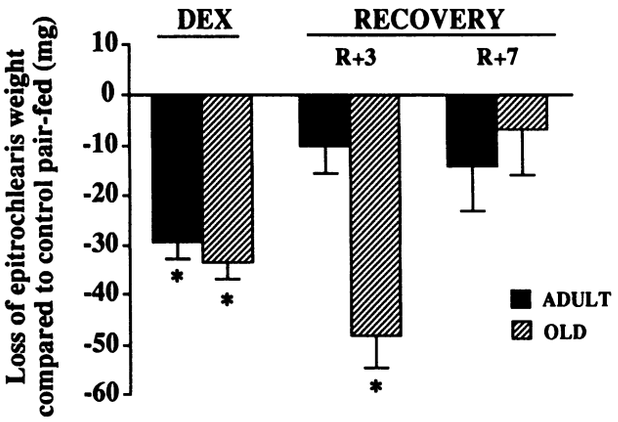

Figure 1. Loss of epitrochlearis muscle mass in adult and old rats during DEX treatment and subsequent recovery, compared with control pair-fed animals. *DEX,* dexamethasone-treated; *R* + 3, 3 d of recovery; *R* + 7, 7 d of recovery. Values are means for 4–6 animals. Vertical bars denote SEM. * $P < 0.05$ vs. control pair-fed values.

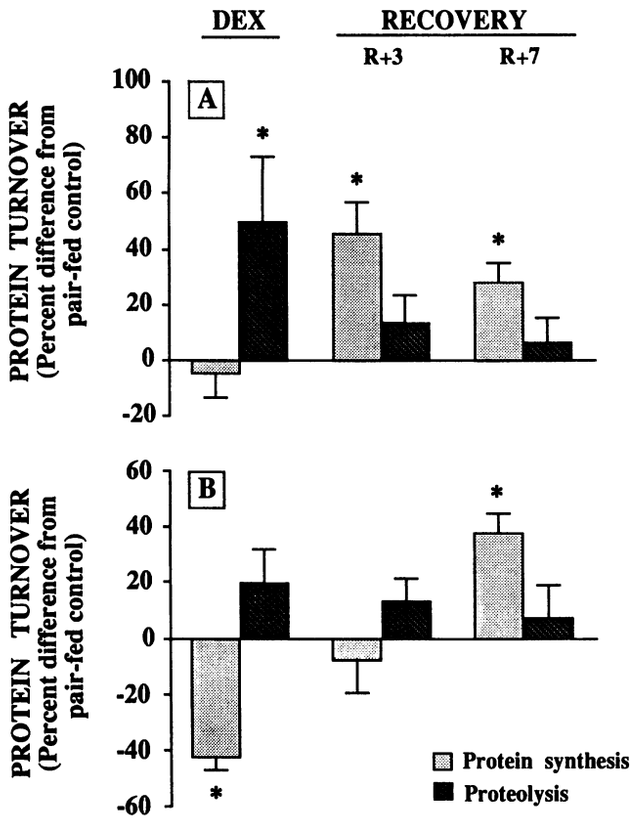

Figure 2. Muscle protein turnover in adult (*A*) and old (*B*) rats during DEX treatment and subsequent recovery. Epitrochlearis muscles were incubated in oxygenated KHB supplemented with glucose, branched chain amino acids, and [^{14}C]phenylalanine. Protein synthesis (nmol of phenylalanine incorporated into protein per mg protein per h) and breakdown (nmol of tyrosine per mg protein per h) are expressed as percent differences from pair-fed controls. *DEX,* dexamethasone-treated; *R* + 3, 3 d of recovery; *R* + 7, 7 d of recovery. Values are means for 4–6 animals. Vertical bars denote SEM. * $P < 0.05$ vs. control pair-fed values.

−33.6±3.2 mg, respectively) (Fig. 1). Three days after dexamethasone withdrawal, the epitrochlearis muscle mass was normalized in adult rats. By contrast, old rats still exhibited a significant reduction in epitrochlearis mass when compared to their pair-fed controls (−48±7 mg) (Fig. 1). Old rats only recovered their epitrochlearis mass after 7 d. Variations in muscle mass reflected changes in protein pools, since protein concentration (mg/mg muscle) was not affected by the DEX treatment (0.138±0.004 and 0.131±0.004 in control and DEX-treated adult rats, respectively; 0.144±0.004 and 0.142±0.002 in control and DEX-treated old rats, respectively). Changes in gastrocnemius/plantaris muscles mass, sensitivities to DEX and patterns of recovery were similar to epitrochlearis in the same group of age (data not shown).

At the end of the DEX treatment period, both adult and old rats showed a significant increase ($P < 0.05$) in blood glucose when compared to their pair-fed controls (168±11 vs. 106±5 mg/dl in adult and 244±11 vs. 125±13 mg/dl in old rats). However, the hyperglycemic effect of DEX was more important in old rats (98 vs. 59%, $P < 0.05$).

Epitrochlearis muscle protein turnover. DEX administration resulted in increased proteolysis (+50%, $P < 0.05$) in adult but not in old rats (Fig. 2). In contrast, protein synthesis remained unchanged after DEX in adult rats, but was strongly depressed in old rats (−42%, $P < 0.05$). Thus, muscle atrophy resulted mainly from enhanced proteolysis in the adult, but from reduced protein synthesis in old rats.

The stimulation of protein breakdown in adult rats and the inhibition of protein synthesis in old animals were not maintained after DEX withdrawal, allowing recovery of muscle proteins. In the adult, proteolysis fell to control values and protein synthesis was stimulated (+45% and +28% at R + 3 and R + 7 respectively, $P < 0.05$). An increase in protein synthesis was also observed in old rats but was only significant at R + 7 (+38%, $P < 0.05$) (Fig. 2). These observations explained the delayed recovery of muscle protein mass in old rats compared with adult animals.

Expression of proteinases or components of proteolytic processes. Recent data indicate that increased mRNA levels for proteinases (14, 43, 47–49), ubiquitin (14, 22, 24, 43, 49) and the 14-kD E2 (37, 43) involved in substrate ubiquitinylation are observed in several muscle wasting conditions. Thus, we analyzed whether enhanced mRNA levels for lysosomal proteases prevailed in the muscles from the DEX-treated animals. The mRNA level for cathepsin D was increased after DEX treatment in both adult and old muscles, but significantly less in old than in adult rats (+272 vs. +383%, respectively, $P < 0.05$) (Table I). Cathepsin D expression was also high during the recovery period at R + 3 (+190%, $P < 0.05$) but was normalized in both groups of rats at R + 7 (Table I). In adult rats, the mRNA levels for cathepsin B followed the same pattern than for cathepsin D during the DEX treatment and the recovery period (Fig. 3). By contrast, no alteration was seen in cathepsin B expression during either the DEX treatment or the recovery period in old rats (Fig. 3).

Since skeletal muscle also contains Ca^{2+}-dependent and ATP-ubiquitin-dependent nonlysosomal proteolytic pathways, we investigated possible changes in mRNA levels for critical components of these processes following DEX treatment. A similar increase in m-calpain expression was seen in both adult and old rat muscles at the end of the DEX treatment (Table I). In both groups, levels of the transcript then slowly decreased

Table I. Effect of Dexamethasone on mRNA Levels for Cathepsin D and m-Calpain in the Muscles from Adult and Old Rats

	Adult			Old		
	DEX	R+3	R+7	DEX	R+3	R+7
Cathepsin D	383±40*	197±30*	103±9	272±14*	193±37*	90±19
m-Calpain	492±96*	253±28*	103±16	530±18*	255±46*	147±25

Muscle total RNA (20 μg) was separated on agarose gel, transferred to nylon membranes and hybridized with the corresponding ^{32}P-dCTP labeled cDNAs. Autoradiographic signals were quantified using a densitometer. The blots were stripped and rehybridized with a ^{32}P-dCTP labeled cDNA encoding GAPDH. Data were corrected for GAPDH mRNA abundance, and expressed as percent differences from pair-fed controls. Values are means±SEM for 3–4 animals. * $P < 0.05$ vs. control pair-fed values.

to control values when rats were allowed to recover (Table I). Northern analysis also revealed that mRNA levels for ubiquitin were significantly increased in DEX-treated rats (Fig. 4). Increased expression for both transcripts of polyubiquitin was similar in adult and old muscles (+233 and +235%, respectively, $P < 0.05$). Ubiquitin mRNAs levels decreased after DEX withdrawal and returned towards control levels by 3 or 7 d in adult and old muscle respectively (Fig. 4). DEX increased the mRNA levels for 14-kD E2 and the C2 subunit of the 20S proteasome in adult muscle (+180 and +240%, respectively, $P < 0.05$) (Fig. 4). Similar data were observed for the C9 proteasome subunit (data not shown). Increased expression of 14-kD E2 and 20S proteasome subunits were transient and rapidly normalized to control levels during the recovery period (Fig. 4). By contrast, DEX treatment had no effect on mRNA levels for 14-kD and the C2 proteasome subunit in old rat muscle (Fig. 4). The mRNA level for the C9 proteasome subunit was also unchanged by DEX treatment in aged muscle (data not shown).

Discussion

Our findings clearly demonstrated that old rats were more sensitive to DEX treatment than adult animals since similar muscle wasting was generated with a shorter treatment period (5 vs. 6 d). The higher hyperglycemic effect generated by DEX in old rats was also in agreement with this observation. In addition, the mechanisms by which DEX induced muscle epitrochlearis atrophy were totally different in adult and old rats (Fig. 2). Indeed, in adult, muscle wasting resulted from increased proteolysis whereas in old rat only decreased protein synthesis was observed. The lack of suppression of protein synthesis with DEX in adult rats is surprising with respect to previous studies (50). However, the data obtained in DEX-treated animals were compared to those recorded in pair-fed control animals. Presumably, the rate of muscle protein synthesis was already depressed in the control animals, because of their very low food intake. Therefore, the data suggest that DEX was only able to further reduce protein synthesis in food-restricted aged animals, but not in the adult.

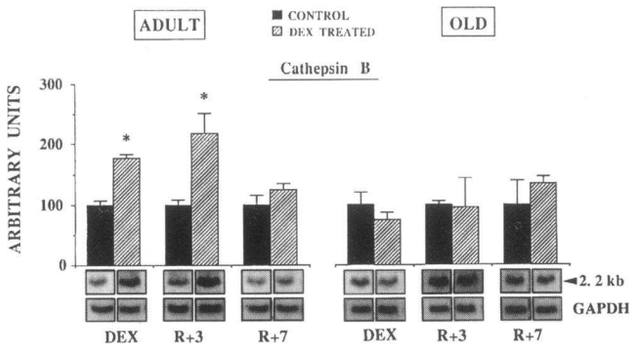

Figure 3. Effect of dexamethasone on mRNA levels for cathepsin B in muscles from adult and old rats. Muscle total RNA (20 μg) was separated on agarose gel, transferred to a nylon membrane and hybridized with a HindIII-linearized digoxigenin-labeled UTP rat cathepsin B riboprobe. Autoradiographic signals were quantified using a densitometer. The blots were stripped and rehybridized with a ^{32}P-dCTP labeled cDNA encoding GAPDH. Data were corrected for GAPDH mRNA abundance, and expressed as percent differences from pair-fed controls. *DEX,* dexamethasone-treated; *R* + 3, 3 d of recovery; *R* + 7, 7 d of recovery. Values are means for 3–4 animals. Vertical bars denote SEM. Representative northern blots are also shown. * $P < 0.05$ vs. control pair-fed values.

After DEX withdrawal, adult rats rapidly restored their muscle protein mass within 3 d whereas in old animals, this phenomenon was delayed to 7 d. An age effect in recovery process was previously observed in others stress situations such as bone fracture in humans (51). Our experiments showed that muscle mass was normalized in adults by both a reduced rate of proteolysis and an increased rate of protein synthesis, whereas in aged animals, only an increased protein synthesis was observed; recovery was thus slowed down. Nevertheless, it is important to note that in our experiments, the absolute increase in protein synthesis observed during the first three days of recovery was the same in adult and old rats suggesting that the ability of protein synthesis to respond to anabolic stimuli was maintained during aging. In accordance with this idea, we previously reported that the ability of amino acids and insulin to stimulate protein synthesis was preserved in old rat muscle (33, 52).

Increased mRNA levels for proteinases (cathepsins, calpains, 20 S proteasome) or cofactors (ubiquitin, 14-kD E2) involved in protein breakdown have been reported in many muscle wasting conditions (reviewed in reference 48). In the present study, we provide the first evidence for increased concomitant expression of lysosomal, Ca^{2+}-dependent and ATP-ubiquitin-dependent proteases following DEX treatment in the adult rat. These findings are not unexpected since a coordinate activation of the ATP-ubiquitin-dependent proteolytic pathway with either lysosomal proteinases (24) or Ca^{2+}-dependent proteinases (43) or both (18, 47) seemed to prevail in different muscle wasting conditions. Presumably the activation of various proteolytic systems functions to eliminate different classes of proteins.

By contrast, DEX did not significantly stimulate proteolysis in old rats after 5 d of treatment, although we cannot rule out

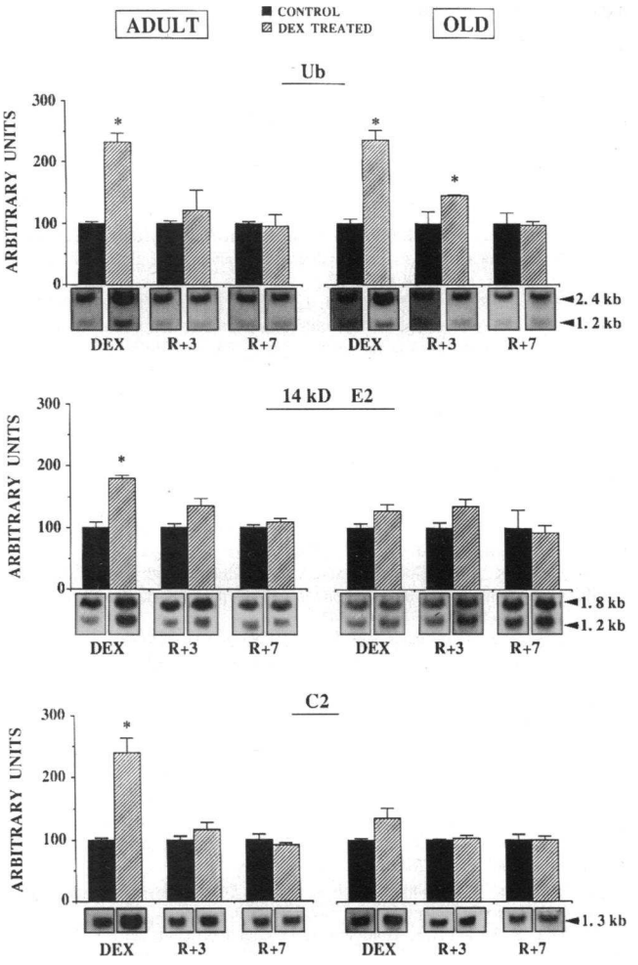

Figure 4. Effect of dexamethasone on mRNA levels for ubiquitin, 14-kD E2 and C2 subunit of the 20S proteasome in the muscles from adult and old rats. Muscle total RNA (20 μg) was separated on agarose gel, transferred to nylon membranes and hybridized with the corresponding ^{32}P-dCTP labeled cDNAs. Autoradiographic signals were quantified using a densitometer. The blots were stripped and rehybridized with a ^{32}P-dCTP labeled cDNA encoding GAPDH. Data were corrected for GAPDH mRNA abundance, and are expressed as percent differences from pair-fed controls. *DEX,* dexamethasone-treated; *R* + 3, 3 d of recovery; *R* + 7, 7 d of recovery. Values are means for 3–4 animals. Vertical bars denote SEM. Representative northern blots are also shown.

an earlier transient effect of DEX. At the time the experiments were performed, DEX failed to stimulate the expression of the 14-kD E2, proteasome subunits and cathepsin B, but not of cathepsin D, m-calpain or ubiquitin. The fact that there was increased mRNA levels for some of these genes indicated that there was no general blockade in the action of glucocorticoids in old rats. Since the increased mRNA levels for m-calpain and cathepsin D in DEX-treated old rats did not affect the overall protein breakdown, these proteinases may play a minor role in the degradation of muscle proteins. Indeed, there is now strong evidence that neither the lysosomal nor the Ca^{2+}-dependent proteinases are involved in the breakdown of myofibrillar proteins (17, 18, 22). The increased ubiquitin mRNA levels in DEX-treated old animals without any change in protein breakdown was also not really surprising since the chicken polyubiquitin gene contains a glucocorticoid responsive element (53). By contrast, the inability of glucocorticoids to stimulate skeletal muscle protein breakdown in old rats could be a consequence of the lack of effect of DEX on mRNA levels for the 14-kD E2 and proteasome subunits. Enhanced expression of these components of the ubiquitin-proteasome proteolytic pathway has been reported in diverse muscle wasting conditions such as starvation (19, 37), denervation atrophy (19), simulated weightlessness (48), cancer (43), acidosis (14, 49), infection (54), and trauma (55). The lack of activation of the ubiquitin-proteasome proteolytic pathway in aged muscle is in accordance with data previously reported in aging human fibroblasts (56). However, it is important to note that modulation of specific mRNA levels may not represent the only regulation point controlling protein breakdown in muscle. For example, activity of the different proteolytic pathways can be regulated by many other mechanisms such as compartmentalization (e.g., autophagy), inhibitors (e.g., calpastatin, the inhibitor of calpains), and post-translational modifications. Therefore, further experiments should test whether or not DEX may affect the activity of the different proteinases in adult and old rat muscle.

The origin of the disappearance of the stimulatory effect of DEX on muscular proteolysis in old rats is unknown. We first hypothetized that glucocorticoid signalling was impaired, i.e. there might be alterations of local regulatory factors that make proteolysis unresponsive to glucocorticoids. This could not arise from glucocorticoid receptors since their expression has been reported to be specifically increased with aging (57). A reduced entry of activated glucocorticoid-receptor complexes was nevertheless reported in the aged rat liver (58). Such an observation in muscle would be consistent with a general impairment of glucocorticoid action, and thus would not explain the specific blockade of gene expression that we observed. The cascade of events linking glucocorticoids to the stimulation of ATP-ubiquitin dependent proteolysis remains to be elucidated. It is presently unknown whether or not the genes of components of the ubiquitin-proteasome proteolytic pathway contain glucocorticoid responsive elements, except for ubiquitin (53). An increased expression of ubiquitin (24) or of ubiquitin and proteasome subunits (14) has been previously reported in rat skeletal muscle following DEX treatment.

Glucocorticoids have a stimulatory effect on muscle proteolysis in young fasted rats (11, 24). This effect sometimes disappears when animals are in a fed state (7, 9). The likely explanation for the different effect of glucocorticoids on proteolysis in fed and fasted animals is that insulin influences the muscle's response to steroids. Indeed, when muscles from fasted adrenalectomized animals are incubated with insulin and glucocorticoids, the increased proteolysis does not occur (11). In addition, insulin has been shown to inhibit the expression of the 14-kD E2 (37). We thus raised the possibility that the disappearance of the stimulatory effect of glucocorticoids on proteolysis in old rats was related to an improved insulin action. This hypothesis seems unlikely since plasma insulin levels were not different in basal conditions (52) and the decrease in food intake associated to the overnight fast before muscle incubation decreased, in our experiment, the hyperinsulinemia generated by DEX to the same level in both adult and old rats (20.2±4.3 and 23.1±0.9 mU/ml, respectively). Furthermore, previous experiments (33, 59) showed that aging was not associated with significant modification of the sensibility of glucose metabolism and protein synthesis to insulin. Concerning muscle proteolysis, data reported by Louard et al. (60) makes definitively this hy-

pothesis unlikely since they showed that the antiproteolytic action of insulin was deteriorated in glucocorticoid-treated subjects.

Our study brings for the first time new insights into the mechanisms possibly underlying muscle wasting during aging. We report here an increased sensitivity of muscle protein turnover to DEX in aging. Since an increase in plasma glucocorticoids was described in old rats (32), this may create a slight but continuous imbalance between muscle protein synthesis and breakdown, generating a loss of proteins over a long period. Furthermore, in states where elevated circulating glucocorticoids levels prevail (stress or pathological conditions), the inability of old rats to catch-up rapidly may result in muscle atrophy, especially when another stress situation is initiated before muscle mass recovery is complete.

In conclusion, we demonstrated that glucocorticoid action on skeletal muscle protein turnover differed markedly between adult and old rats. Glucocorticoids induced more rapidly muscle wasting in aging and the recovery of muscle mass was impaired. These responses to glucocorticoids in old rats may be involved in the emergence of muscle atrophy with advancing age. Furthermore, glucocorticoids stimulated proteolysis in adult but depressed protein synthesis in aged animals. The disappearance of the stimulatory effect of glucocorticoids on protein breakdown in aging occurred along with a loss of the ability of steroids to enhance the expression of the 14-kD E2 and of subunits of the 20 S proteasome. Thus, these data clearly support a major role of the ATP-ubiquitin-dependent proteolytic pathway in the control of muscle mass and may contribute to explain the accumulation of abnormal proteins in aged muscle (61).

Acknowledgments

We would like to thank Dr. Keiji Tanaka (Institute for Enzyme research, Tokushima, Japan) for the gift of the plasmids encoding the rat proteasome subunits, Dr. Simon S. Wing (McGill University, Montréal, Canada) for providing us with the cDNA of the rat 14-kD E2, and Dr. Susan E. Samuels for helpful discussions. This study was supported in part by research grants from the French Ministère de l'Enseignement Supérieur et de la Recherche, and the Institut National de la Recherche Agronomique.

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