Research paper

Insulin-like growth factor-1 restores dexamethasone-induced heart growth arrest in rats: the role of the ubiquitin pathway

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ABSTRACT

OBJECTIVE: In skeletal muscle, glucocorticoids induce catabolism and proteolysis which is accomplished via the ubiquitin (Ub) proteolytic pathway. Cardiac muscle is a striated muscle which, compared to skeletal muscle, more abundantly expresses components of the Ub pathway, thus suggesting an important role for this pathway in heart physiology. The aim of our study was to explore the role of the Ub pathway in heart muscle physiology. **DESIGN:** We treated rats for three days with a pharmacologic dose of dexamethasone (DEXA) 0.5mg/100g body weight (BW). An attempt was also made to counteract the DEXA effect by co-treatment with insulin-like growth factor-1 (IGF-1; 0.35mg/100g BW). **RESULTS:** DEXA treatment caused a 7.8% decrease in heart weight compared to control ($p < 0.05$) and also increased heart tissue levels of the ubiquitin-conjugating enzyme E2 and the 20S proteasome protein. Myofibrillar proteins degraded by the ubiquitin pathway (α -actin, myoglobin, and troponin 1) were all decreased by DEXA, while ubiquitinated forms of α -actin were increased. Co-treatment with IGF-1 completely prevented DEXA-induced decrease in heart weight, an effect which was accompanied by decreased heart tissue levels of several ubiquitinated proteins including α -actin, the 20S proteasome protein, E2-14kDa mRNA, and C-3 proteasome subunit mRNA, while the levels of non-ubiquitinated α -actin, myoglobin, and troponin 1 were all partially restored. **CONCLUSION:** These results demonstrate that DEXA activates the ubiquitin proteolytic pathway in the heart and that IGF-1 efficiently counteracts this effect. Our findings reveal a possible mechanism for the anti-proteolytic actions of IGF-1 and its cardioprotective role involving the Ub pathway.

Key Words: Apoptosis, Dexamethasone, Glucocorticoids, Heart, IGF-1, Ubiquitin, Ubiquitin pathway

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INTRODUCTION

Glucocorticoids cause muscle atrophy in humans and animals by decreasing protein synthesis and increasing protein degradation.¹ The ubiquitin (Ub) proteasome pathway is considered to be the principal pathway of proteolysis of myofibrillar proteins in skeletal muscle during glucocorticoid treatment.²⁻⁵ This pathway carries out proteolysis of the ubiquitinated proteins targeted for degradation by sequential preparatory steps involving Ub, Ub-activating enzymes (E1), Ub-conjugating enzymes (E2), and Ub-ligases (E3). Briefly, Ub is activated by an E1 enzyme (requiring ATP), it is then transferred to one of the E2 enzymes, and finally, with the help of the E3 ligases, Ub is linked to the protein targeted for degradation. These steps are repeated and several Ub molecules are attached to the protein targeted for degradation. Finally, these steps are followed by proteolysis of the ubiquitinated protein by proteasomes (Figure 1).⁶ The Ub-pathway is important not only for the degradation of muscle proteins but also for the degradation of rate-limiting enzymes, proteins involved in the cell

cycle (cyclins) and the removal of abnormal proteins.⁷ In heart muscle, mRNAs of different components of the Ub-pathway and proteasome activity are more abundant than in skeletal muscle⁸ and decline with age⁹ suggesting a key role for heart muscle turnover. Studies showing that the E3-ligases are critical for maintenance of heart mass further support the concept that the Ub pathway plays an important role in heart physiology.¹⁰⁻¹² The Ub pathway has also been implicated in various conditions affecting the heart such as diabetes, dilated cardiomyopathy, oxidative stress, and ischemia-reperfusion injury.^{8,13-15}

Animal studies suggest that cardiac muscle is more resistant to the catabolic effects of glucocorticoids compared to skeletal muscle.¹⁶ Whereas the catabolic actions of glucocorticoids have been extensively studied in skeletal muscle, there are few reports directly addressing this issue in heart muscle. Glucocorticoid treatment has been reported to either increase or decrease, or else have no effect on protein degradation in heart muscle.¹⁷⁻²⁰ Differences in the methodology of measuring protein degradation, glucocorticoid

Figure 1. The ubiquitin proteasome pathway (Ub-pathway).⁶ Ub is activated by an E1 enzyme requiring ATP. It is subsequently transferred to one of the E2 enzymes and finally, with the help of the E3 ligases, Ub is linked to the protein targeted for degradation. These steps are repeated and several Ub molecules are attached to the protein targeted for degradation. Polyubiquitinated proteins are recruited by the barrel-shaped 26S proteasome which is formed by the 20S and 19S particles containing different subunits. The 19S particle possesses Ub-dependent proteolytic potential to remove Ub from the substrate for recycling, and ATPase activity to unfold the substrate protein which will be degraded into small peptides (yellow dots) by the 20S core. The 20S core is formed by different proteasome subunits. Deubiquitinating enzymes (DUB) counteract the activity of E3 and cleave Ub for recycling at the 26S proteasome (with permission).

dosing regimen, and animal age may account for this variability in reported data. In animal models of sepsis and burn injury, in which the endogenous production of glucocorticoids is increased, components of the Ub pathway have been reported not to be up-regulated.^{21,22}

IGF-1 is an anabolic peptide which promotes growth not only by increasing cell proliferation and survival but also by increasing protein synthesis and decreasing protein breakdown.^{23,24} The anti-proteolytic actions of IGF-1 are considered to be mediated, at least partially, through the Ub-pathway. As an example, in models of catabolism, such as burn injury and glucocorticoid exposure, IGF-1 treatment has been shown to suppress Ub, E2 enzymes, E3 ligases, and subunits of the proteasomes.²⁵⁻²⁷ At the molecular level, these actions of IGF-1 are mainly mediated through the PI3-kinase/akt pathway which inhibits the transcription factor FOXO, an important stimulator of several E3-ligases.^{24,28} IGF-1 promotes heart growth and its administration increases cardiac mass and contractility.²⁹⁻³¹ Furthermore, IGF-1 increases the synthesis of myocardial proteins in the rat heart like the heavy chain myosin,³² but it is not known whether it also exerts anti-catabolic actions by decreasing protein breakdown.

While, as mentioned above, there has been extensive examination in skeletal muscle of the catabolic actions of glucocorticoids, no studies currently exist that directly deal with this subject in heart muscle. The present study was designed to investigate the effects of pharmacologic doses of dexamethasone (DEXA) on heart muscle focusing on different components of the Ub-pathway. We also aimed to study if co-treatment with IGF-1 could prevent DEXA-induced catabolism and associated alterations of the Ub-pathway.

MATERIAL AND METHODS

Reagents

Digoxigenin (DIG)-11-UTP, DIG chemiluminescent detection reagents, DNA polymerase, RNA polymerases, positively charged nylon membranes, and a kit for immunoprecipitation were purchased from Roche Diagnostics Scandinavia AB (Bromma, Sweden). Recombinant IGF-1 was a gift from Pfizer. The puri-

fication system for PCR products was obtained from Qiagen (Chatsworth, CA). TPIzol for RNA extraction was purchased from Life Technologies, Inc. (Grand Island, NY). PVDF membranes, protein molecular standards, and the ECL^{plus} chemiluminescent system for Western immunoblots were from Amersham Life Sci. (Arlington Heights, IL). Goat polyclonal antibodies against α -actin, troponin 1, and myoglobin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mouse monoclonal antibody FK1 which recognizes only polyubiquitinated proteins was purchased from Biomol (Enzo Life Sciences International Inc., Plymouth Meeting, PA). The E2-14kDa antibody was kindly offered by Dr Wing SS (McGill, Montreal, Quebec, Canada) and the antibody against the α subunit of 20S proteasome (clone MCP231) which recognizes subunits α 1, α 2, α 3, α 5, α 6, and α 7 was from Biomol (Enzo Life Sciences International Inc.). The MCP231 antibody was marketed by Affiniti Research Products Limited, a company which was later merged with Biomol, the company from which we bought the same antibody. Biomol was recently fully integrated into Enzo Life Sciences.

Animals and experimental design

Five-week-old male Sprague-Dawley rats (150g) were exposed to 12-h light, 12-h dark cycles, and fed ad libitum. After an adaptation period, 15 animals were randomly divided into 3 groups. One group received DEXA (DEXA group: 0.5mg/100g body weight (BW) per day, for 3 days) subcutaneously (SC). Another group received DEXA and IGF-1 in combination (DEXA/IGF-1 group: 0.5mg/100g BW/day DEXA and 0.35mg/100g BW/day IGF-1, SC, divided into 2 daily doses, for 3 days), while a third group (control group) received a single daily injection of vehicle for 3 days.

At the end of the experimental period, the animals were sacrificed by intraperitoneal (IP) injection of phenobarbital and their hearts were excised, weighed, flash-frozen in liquid nitrogen, and stored at -80°C . The experimental protocol was approved by the local Ethics Committee at the Karolinska Institute.

Northern hybridization analysis

Northern hybridization analysis was performed as previously reported.¹⁴ Briefly, 15 μ g of total RNA

extracted from heart (TRIzol, Life Technologies) was fractionated on a 1.2% agarose gel containing 2.2 M formaldehyde, transferred overnight onto positively charged nylon membranes, fixed with UV crosslinking, and stained with methylene blue to ensure uniform loading and transferring. The membranes were hybridized overnight at 68°C in hybridization buffer, washed, and then subjected to chemiluminescent detection. Chemiluminescent signals were quantified densitometrically. Northern hybridization analysis was performed for the Ub, E2-14kDa enzyme, and C-3 proteasome subunit mRNAs by using the corresponding riboprobes as previously reported.^{26,27}

Western immunoblotting and immunoprecipitation

Total protein was extracted from the hearts with the modified RIPA buffer²⁶ containing a protease inhibiting cocktail with phenylmethylsulfonyl fluoride (Roche Diagnostics Scandinavia AB). Protein concentrations were measured via the Bradford method (BioRad, Hercules, CA). Equal amounts of protein were fractionated by 12% (or 8% for Ub) SDS-PAGE under reducing conditions. Resolved proteins were electrophoretically transferred to PVDF membranes and blocked overnight at 4°C in TBS-T (TBS, 10mM Tris and 150mM NaCl, pH 7.5 with 0.1% Tween) containing 5% skim milk. Membranes were then incubated with primary antibodies (Ub: 1:750, α -actin 1:2500, troponin 1 1:2000, myoglobin 1:2500, E2-14kDa 1:3000, 20S proteasome 1:3000) in TBS-T containing 0.5% milk (E2-14kDa in TBS-T containing BSA) at room temperature for 1h, washed in TBS-T several times, and incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin (Transduction Laboratories, San Diego, CA) at 1:10,000 dilution or anti-goat (Santa Cruz Biotechnology Inc, Santa Cruz, CA) at 1:3000 dilution. The antigen-antibody complexes were then detected by chemiluminescence. After development of films, blots were stained with Coomassie blue to ensure equal loading of total protein. In addition, in order to evaluate further the equal loading of total proteins, some blots were re-incubated with an antibody for the housekeeping protein GADPH (Santa Cruz Biotechnology Inc.).

Immunoprecipitation was carried out according to the manufacturer's protocol (Roche Diagnostics Scandinavia AB). Five hundred micrograms of total

heart protein were precleaned with protein G-agarose (50 μ L agarose for 2h in rotor at 4°C, and then another 50 μ L of agarose overnight). Beads were pelleted by gravity sedimentation and supernatants were removed. In each sample, 1 μ g of a goat polyclonal antibody against α -actin was added and incubated for 1h at 4°C, then 50 μ L of protein-G suspension was added and incubated overnight at 4°C. Complexes were collected by gravity sedimentation, washed twice for 20min at 4°C on a rock platform with a buffer containing 50mM Tris-HCL, pH 7.5, 150mM NaCL, 0.1% Nonidet P40, 0.5% sodium deoxycholate and protease inhibitors, and another 2 times in washing buffer containing 50mM Tris-HCL, pH 7.5, 500mM NaCL, 0.1% Nonidet P40, 0.05% sodium deoxycholate, and then once in a buffer containing 10mM Tris-HCL, pH 7.5, 0.1% Nonidet P40 and 0.5% sodium deoxycholate. Immunoprecipitated proteins were dissolved in loading buffer and denatured by heating at 100°C. An aliquot of immunoprecipitated proteins was analyzed by SDS-polyacrilamide electrophoresis and immunoblotted with an antibody against Ub (UG9510, Biomol, Enzo Life Sciences International Inc.). Normal goat serum instead of heart protein was used as a negative control during immunoprecipitation.

Statistics

All values are presented as median and range. Because of the small number of animals in each group, non-parametric one-way analysis of variance (ANOVA) was applied (Kruskal-Wallis test). Levels of significance were analyzed with Dunn's comparison post test and p values <0.05 were considered significant. The statistical software program GraphPad Prism, version 5.02, was used.

RESULTS

Body weight

Rats treated for 3 days with DEXA alone lost weight by 12.2% (range 10.6-13.3%) in contrast to control rats that gained weight by 16.2% (range 14.7-18%) compared to their initial body weight (Table 1). Rats treated with the combination of IGF-1 and DEXA also lost weight (p<0.5 vs. control) but to a lesser degree than rats treated with DEXA alone (6.3% (range 4.7-7.8%) vs. 12.2% (range 10.6-13.3%) weight loss; p<0.015 vs. DEXA).

Table 1. Data obtained in animals treated with either dexamethasone (DEXA) or the combination of DEXA with insulin like growth factor-1 (DEXA/IGF-1) and in controls. Median values (range)

	Controls	DEXA	DEXA/IGF-1
Initial BW (g)	167.5 (161-168)	168.0 (165-175)	167.5 (163-170)
Final BW (g)	192.2 (190-195.2)	146.9 (143.5-154.8) ^a	156.7 (155.4-157.2) ^{c,d}
Change in BW (% of base line)	+16.2 (14.7-18.0)	-12.2 (10.6-13.3)	-6.3 (4.7-7.8) ^b
Heart weight (g)	0.77 (0.73-0.82)	0.71 (0.69-0.73) ^c	0.81 (0.76-0.84) ^b
Heart weight (% of BW)	0.40 (0.39-0.42)	0.48 (0.47-0.49) ^c	0.52 (0.49-0.54) ^a
Change in heart weight (% of control)		- 7.8 ± 1.2	+ 5.2 ± 0.9 ^b

^aP <0.01 vs. control, ^bP <0.01 vs. DEXA, ^cP <0.05 vs. control, ^dP <0.05 vs. DEXA, BW: body weight.

Heart weight

Following 3 days treatment with DEXA, heart weight was significantly lower when compared to vehicle treated control rats [0.71g (range 0.69-0.73) vs. 0.77g (range 0.73-0.82g); $p < 0.05$]. Co-administration of IGF-1 completely prevented DEXA-induced reduction in cardiac weight [0.81g (range 0.76-0.84g) heart weight in DEXA/IGF-1 vs. 0.71g (range 0.69-0.73g) in DEXA only; $p < 0.01$]. Moreover, heart weight in DEXA/IGF-1 rats showed a tendency to exceed that in control animals (Table 1).

Ubiquitin (Ub) mRNA and protein levels in heart

DEXA treatment caused a small but significant decrease of the 2.8 and 1.2Kb Ub transcripts (68% of control, range 60-76%; average of both transcripts; $p < 0.01$), an effect which could not be prevented by the co-administration of IGF-1 and DEXA (74% of control; range 68-82%). In contrast to mRNA levels, DEXA caused a significant increase in the total amount of ubiquitinated proteins above 60kDa (207% of control level; range 190-230%; $p < 0.01$), an effect which was prevented by co-treating the animals with IGF-1 and DEXA (125% of control; range 95-140%; $p < 0.05$ vs. DEXA only; Figure 2A). These protein data were confirmed using a second anti-Ub antibody (UG9510, Biomol, Enzo Life Sciences International Inc.) that recognizes Ub-conjugated proteins.

Ub-conjugating enzyme E2 in heart

In contrast to Ub mRNA levels, DEXA treatment increased both the 1.8 and 1.2Kb transcripts of the E2-14kDa enzyme (218% of control; range 187-270%; average of both transcripts; $p < 0.01$ vs.

control), an effect which was significantly prevented when animals were co-treated with IGF-1 and DEXA (142% of control; range 123-157%; $p < 0.05$ vs. DEXA only, $p > 0.05$ vs. control). When measuring E2-14kDa protein levels it was found that neither DEXA nor DEXA/IGF-1 treatment had any significant effect.

Heart proteasome expression

The mRNA level for the proteasome subunit C-3 was unaffected by DEXA treatment (90, range 80-98% of control, $p > 0.05$), whereas IGF-1 significantly suppressed it (45% of control, range 39-51%, $p < 0.05$ vs. DEXA). Western immunoblots with the 20S antibody (recognizing α -subunits 1, 2, 3, 5, 6, and 7) revealed a significant increase in proteasome protein level by DEXA (140% of control; range 134-160%, $p < 0.05$ vs. control), whereas the combined treatment with DEXA and IGF-1 completely prevented this increase (84% of control; range 68.3-107.9%, $p < 0.01$ vs. DEXA only; Figure 2B).

Heart expression of α -actin, myoglobin, and troponin I

DEXA treatment markedly reduced the α -actin protein level (57% of control; range 50-63%, $p < 0.01$), whereas IGF-1 partially restored it (78% of control; range 65-93%, $p < 0.05$ vs. control, and $p > 0.05$ vs. DEXA), (Figure 3A). The myoglobin protein level was decreased by DEXA (48% of control; range 38-58%, $p < 0.01$), whereas co-administration of DEXA and IGF-1 partially restored it (78% of control; range 68-81%, $p < 0.05$ vs. control group, and $p > 0.05$ vs. DEXA), (Figure 3B). Also the troponin 1 level was decreased by DEXA administration (47% of control; range 35-59%; $p < 0.01$), whereas IGF-1 and DEXA

Figure 2. A: The effects of DEXA and IGF-1 on ubiquitinated proteins as assessed by western immunoblotting. Bands above 60kDa were quantified. Total ubiquitinated proteins were increased by DEXA compared to the control group (** $p < 0.01$), while co-administration of IGF-1 reduced them significantly (* $p < 0.05$ vs. DEXA). **B:** The effect of DEXA and IGF-1 on α proteasome subunits 1, 2, 3, 5, 6, and 7 was assessed by western immunoblotting. DEXA increased the protein levels of proteasome subunits significantly (* $p < 0.05$ vs. control, and ** $p < 0.01$ vs. DEXA/IGF-1) and the addition of IGF-1 restored them to control levels.

in combination partially restored troponin 1 (76% of control, range 66-80%; $p < 0.05$ vs. control group, and $p > 0.05$ vs. DEXA) (Figure 3C).

Ubiquitinated forms of α -actin in heart

To investigate whether the decrease in α -actin in heart was a result of increased activation of the Ub-pathway, we immunoprecipitated α -actin and then

Figure 3. The effects of DEXA and DEXA/IGF-1 on myofibrillar proteins as assessed by western immunoblotting. **A:** DEXA caused a significant decrease in α -actin expression (** $p < 0.01$ vs. control) and IGF-1 partially restored it (* $p < 0.05$ vs. control). **B:** Myoglobin was decreased by DEXA (** $p < 0.01$) and the addition of IGF-1 partially restored it (* $p < 0.05$ vs. control). **C:** Troponin 1 was also decreased by DEXA (** $p < 0.01$ vs. control) and the addition of IGF-1 partially restored it (* $p < 0.05$ vs. control).

performed immunoblotting for Ub. These experiments revealed that DEXA caused a several-fold increase in ubiquitinated forms of α -actin (865% of control; range 790-988%, $p < 0.001$), and the combined treatment with DEXA and IGF-1 partially prevented this increase (357% of control; range 303-394%, $p < 0.01$ vs. DEXA alone), (Figure 4). Ubiquitinated forms of α -actin were above 60kDa with a major band at 70kDa. Immunoprecipitation with normal goat serum instead of heart protein was used as a negative control and gave no signal in the area of the ubiquitinated forms of α -actin (data not shown).

Figure 4. The effect of DEXA and IGF-1 on ubiquitinated α -actin. Heart lysate from control, DEXA, and DEXA/IGF-1 treated animals was subjected to immunoprecipitation for α -actin followed by western immunoblotting for ubiquitin. DEXA treatment increased significantly ubiquitinated forms of α -actin (** $p < 0.001$ vs. control) with a major band above 60kDa. Co-administration of IGF-1 to DEXA treated rats significantly reduced the ubiquitination of α -actin (** $p < 0.01$ vs. DEXA alone).

DISCUSSION

We here report that pharmacological doses of DEXA inhibit heart growth in rats, an effect associated with decreased levels of the myofibrillar proteins α -actin, myoglobin, and troponin 1 and increased levels of the 20S proteasome and ubiquitinated proteins. Co-administration of DEXA and IGF-1 restored heart weight and the up-regulated components of the

Ub-pathway. Our results clearly show that DEXA activates different components of the Ub-pathway in heart muscle and that IGF-1 counteracts these effects.

Cardiac muscle has been considered more resistant to the catabolic actions of glucocorticoids compared to skeletal muscle.^{8,33} We herein provide evidence that the Ub-pathway is up-regulated in heart muscle after DEXA administration. We examined three different steps in the Ub-pathway: Ub, ubiquitin conjugating enzyme (E2-14kDa), and proteasomes. Our results show that all these components are affected by DEXA treatment, mainly and most significantly at the protein level. The accumulation of ubiquitinated proteins is a strong indication that protein ubiquitination is accelerated in heart after DEXA treatment suggesting increased proteolysis. If the Ub-pathway is activated, the myofibrillar proteins α -actin, myoglobin, and troponin 1, reported to be degraded by the Ub-pathway,³⁴⁻³⁷ would be decreased after DEXA treatment. Indeed, we found that all these proteins decreased post DEXA and partially restored by the addition of IGF-1. Finally, immunoprecipitation experiments directly confirmed that the decrease in α -actin after DEXA treatment was accompanied by a significant increase in ubiquitinated forms of α -actin. Ubiquitination is an essential step for the degradation of proteins targeted to be degraded through the Ub-pathway. The up-regulation of the Ub-pathway caused by DEXA treatment and the associated decrease in myofibrillar proteins lead to increased ubiquitination of α -actin, the final step for degradation of proteins by the Ub-pathway. Altogether, our results clearly show that DEXA activates the Ub-pathway in heart.

Although we made an attempt, by using protease inhibitors, to inhibit endogenous deubiquitinases, the enzymes which cleave polyubiquitin chains, our data might have been affected by any remaining deubiquitinase activity. In any case, the interpretation of our results should not have been affected since any remaining deubiquitinase activity does not differ between groups. Furthermore, any incomplete inhibition of deubiquitinases would just lead to an underestimation of the ubiquitination of proteins by DEXA treatment.

In DEXA treated rats, we found a 7.8% reduction in heart weight compared to controls, whereas body weight was decreased by as much as 26% compared

to the control group. In addition, in the same experimental model we previously found that DEXA causes a 24% reduction of the gastrocnemius muscle weight.²⁶ The fact that the heart weight expressed as a percentage of total body weight was higher in DEXA treated animals compared to controls shows that this treatment results in a relatively higher cardiac mass compared to control animals. We have previously shown that in skeletal muscle of the same experimental model, DEXA induces greater increases in Ub-protein conjugates (3-fold increase) and mRNAs encoding Ub, E2-14kDa, and proteasomes subunits (12-, 6-, and 4-fold increase, respectively) compared to heart.^{26,27} This could indicate a more robust activation of the Ub-pathway and subsequently a higher rate of proteolysis in skeletal muscle compared to heart muscle. The milder effect of DEXA on heart muscle weight compared to skeletal muscle could be the result of a milder activation of the Ub-pathway and proteolysis in combination with positive effects on heart tissue such as increased cell survival. Indeed, glucocorticoids have been demonstrated to have anti-apoptotic effects in heart muscle tissue as well as in cultured cardiomyocytes.³⁸⁻⁴⁰ Altogether, the above findings suggest that the heart is relatively resistant to the catabolic actions of DEXA compared to skeletal muscle.

Co-administration of IGF-1 completely prevented DEXA-induced growth arrest of the heart. In rats treated with DEXA plus IGF-1, heart weight had a tendency to be even higher than in control animals. As DEXA plus IGF-1 only partially preserved body weight, this possibly indicates that the treatment potentially leads to an increased percentage of heart weight per body weight compared to the control group. IGF-1 is an anabolic peptide and promotes growth not only by increasing proliferation, cell survival, and protein synthesis but also by decreasing protein breakdown.^{25,41-43} In models of catabolism, such as fasting,⁴⁴ burn injury,⁴⁵ and glucocorticoid treatment,²⁶ IGF-1 treatment decreases mRNA abundance of components of the Ub-pathway and therefore the Ub-proteasome pathway is considered as a mediator of the anti-proteolytic actions of IGF-1. Furthermore, IGF-1 is known to down-regulate the expression of the ubiquitin ligases atrogin-1 and MuRF-1, both im-

portant in skeletal muscle for the targeting of proteins to be degraded through the Ub-pathway.^{22,24,46} This is accomplished by the inhibition of the transcription factor FOXO, a major stimulant of atrogin-1 and MuRF-1.^{24,47,48}

We demonstrated that IGF-1 regulates not only mRNAs encoding components of the Ub-pathway but also decreases the levels of Ub-conjugated proteins, the 20S proteasome protein and, most importantly, the ubiquitinated forms of α -actin. In addition to its anti-proteolytic actions, IGF-1 has also been reported to increase the synthesis of cardiac myosin heavy chain and actin in rats³² and therefore we cannot exclude that the observed increases in actin are only due to decreased proteolysis.³² It is noteworthy that the effect of IGF-1 administration was more pronounced in preserving heart weight than body weight. In normal rats receiving IGF-1 increased protein synthesis, cellular hypertrophy, and survival leading to cardiac hypertrophy and improved cardiac performance has been observed.³⁰ In addition, IGF-1 exerts cardioprotective effects and improves cardiac function in different animal models with cardiac infarction and heart failure.⁴⁹⁻⁵⁵ Since in this setting the Ub-pathway has been implicated,⁵⁶⁻⁵⁹ one could hypothesize that the cardioprotective effects of IGF-1 detected in those conditions are mediated not only through increased cell survival and protein synthesis but also through regulation of the Ub-pathway.

We conclude that high-dose DEXA treatment in rats leads to an arrest of heart growth with a concurrent activation of the ubiquitin proteolytic pathway and subsequent degradation of myofibrillar proteins. Co-treatment with IGF-1 prevented the DEXA-induced suppression of heart growth and also the activation of components of the Ub-pathway as well as the ubiquitination of actin. Our findings suggest an additional mechanism for IGF-1 in cardioprotective action, namely through regulation of the ubiquitin pathway in the heart.

DECLARATION OF INTEREST

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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