Steroids 107 (2016) 30–36

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Time-course changes of catabolic proteins following muscle atrophy induced by dexamethasone

Anderson G. Macedo [a], André Luis O. Krug [a], Lidiane M. Souza [b], Aline M. Martuscelli [a], Paula B. Constantino [a], Anderson S. Zago [b], James W.E. Rush [c], Carlos F. Santos [d], Sandra L. Amaral [a,b,*]

[a] Joint Graduate Program in Physiological Sciences, PIPGCF UFSCar/UNESP, Department of Physiological Sciences, Federal University of São Carlos – UFSCAR, São Carlos, Brazil
[b] Department of Physical Education, Universidade Estadual Paulista – UNESP, Bauru, Brazil
[c] Department of Kinesiology, Faculty of Applied Health Sciences, University of Waterloo, Ontario, Canada
[d] Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, USP, Bauru, Brazil

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 9 December 2015
Accepted 21 December 2015
Available online 28 December 2015

Keywords:
Skeletal muscle
Glucocorticoids
Flexor hallucis longus muscle
MuRF-1

ABSTRACT

This study was designed to describe the time-course changes of catabolic proteins following muscle atrophy induced by 10 days of dexamethasone (DEX). Rats underwent DEX treatment for 1, 3, 5, 7 and 10 days. Body weight (BW) and lean mass were obtained using a dual energy X-ray absorptiometry (DEXA) scan. Muscle ringer finger1 (MuRF-1), atrogin-1 and myostatin protein levels were analyzed in the tibialis anterior (TA), flexor hallucis longus (FHL) and soleus muscles. DEX treatment reduced lean mass since day-3 and reduced BW since day-5. Specific muscle weight reductions were observed after day-10 in TA (−23%) and after day-5 in FHL (−16%, −17% and −29%, for days 5, 7 and 10, respectively). In TA, myostatin protein level was 36% higher on day-5 and its values were normalized in comparison with controls on day-10. MuRF-1 protein level was increased in TA muscle from day-7 and in FHL muscle only on day-10. This study suggests that DEX-induced muscle atrophy is a dynamic process which involves important signaling factors over time. As demonstrated by DEXA scan, lean mass declines earlier than BW and this response may involve other catabolic proteins than myostatin and MuRF-1. Specifically for TA and FHL, it seems that myostatin may trigger the catabolic process, and MuRF-1 may contribute to maintain muscle atrophy. This information may support any intervention in order to attenuate the muscle atrophy during long period of treatment.

1. Introduction

Muscle wasting is a common side-effect after chronic treatment with corticosteroids or desnervation [1–4]. Muscle atrophy is normally maintained via a tight control of signaling processes involved in both synthesis and degradation of muscle proteins [5,6], but the time-course of molecular signaling mechanisms involved in this response is not completely established.

Previous studies have shown that myostatin, atrogin-1 and MuRF-1 could be involved in muscle atrophy induced by chronic treatment with DEX, however, it is unclear if these proteins respond equally to DEX treatment or if there is a time-dependent effect. Gilson et al. [7] have suggested that myostatin has a crucial role on DEX-induced muscle atrophy, since myostatin-knockout mice did not present muscle atrophy induced by 10 days of DEX treatment. However, Ma et al. [8] have previously demonstrated that myostatin mRNA and protein level were pronounced after 5 days of DEX treatment, but the overexpression was not sustained after 10 days.

Much attention has been given to MuRF-1 and atrogin-1 since there is substantial evidence suggesting that muscle proteolysis may result from ubiquitin–proteasome system activation [1,6,9–11]. We have recently demonstrated that MuRF-1 but not atrogin-1 protein level is increased in skeletal muscle after 10 days of DEX treatment [4]. This result is supported by Baehr et al. [12] who have demonstrated an essential role of MuRF-1 in

Abbreviations: DEX, dexamethasone; C, control; CEUA, Committee for Ethical Use of Animals; DEXA, dual energy X-ray absorptiometry; MuRF-1, muscle ring finger1; Atrogin-1, muscle atrofic F-box; TA, tibialis anterior muscle; FHL, flexor hallucis longus muscle; SOL, soleus muscle; BW, body weight; IRS-1, insulin receptor substrate; GLUT-4, glucose transporter; AKT, serine/threonine protein kinase B; TGF-β, growth stimulating factor β; FOXO, Forkead transcription factors; PMSF, phenylmethylsulfonyl fluoride; IGF-1, insulin-like growth factor-1; mTOR, mammalian target of rapamycin.

* Corresponding author at: Department of Physical Education, Science Faculty, UNESP – Universidade Estadual Paulista, Bauru, SP, Brazil.
E-mail address: slamaral@fc.unesp.br (S.L. Amaral).

http://dx.doi.org/10.1016/j.steroids.2015.12.016
0039-128X/

DEX-treated animals, since a preventive effect was observed on muscle atrophy after glucocorticoid treatment in MuRF-1 knockout mice. Other authors, on the other hand, have shown an increase in atrogin-1 mRNA after DEX treatment; however, these studies used high doses compared with ours. Currently, there is no consensus about the exact mechanism responsible for muscle atrophy induced by DEX treatment. It seems that some proteins may be responsible for triggering this effect whereas others may be responsible for the maintenance of muscle atrophy. Therefore, the aim of this study was to describe the time-course changes of catabolic protein following experimental muscle atrophy induced by 10 days of DEX treatment.

2. Experimental

2.1. Animals

Adult male Wistar rats (200–250 g) were obtained from Center for Research and Production Facilities of UNESP (Botucatu, SP, Brazil) and housed in group cages (5 in each) at the Animal Facility Maintenance from Faculty of Science, UNESP of Bauru. All rats were maintained under controlled environmental conditions (12 h dark–light cycle, 22 °C of temperature) with standard diet (Biobase, Brazil) and water *ad libitum*. All methods were approved by the Committee for Ethical Use of Animals (CEUA) of Universidade Estadual Paulista – UNESP, Araçatuba (approved protocol #2012-02253).

Rats were divided into two groups: DEX, which received DEX treatment for 1, 3, 5, 7 or 10 days (DEX, Decadron®, 0.5 mg/kg of body weight, *i.p*, $n = 40$) and control animals, who received saline during the same period (C, $n = 42$). DEX and saline injections were daily given at the same time (7:00–8:00 h a.m.). Body weight was daily measured during DEX treatment.

2.2. Dual energy X-ray absorptiometry (DEXA) method

Measurements of body weight and lean mass of rats were obtained using a dual energy X-ray absorptiometry scan (DEXA, HOLOGIC, WI, USA), which is considered the standard clinical method to evaluate total body composition. Each anesthetized rat was positioned ventrally with arms separated from the trunk (Fig. 1, panel A) and the whole body was scanned by DEXA, using a specifically designed software version for small animals (Hologic Apex Software, version 3.2, USA).

2.3. Blood glucose determination

After an overnight fasting (12 h), blood samples were obtained from the tail puncture and the blood glucose was analyzed using glucose testing strips and a digital glucometer (One Touch Ultra – Johnsons & Johnsons®, USA).

2.4. Tissue harvesting

Tibialis anterior (TA), flexor hallucis longus (FHL) and soleus (SOL) muscles were removed and weighed just after euthanasia by an overdose of anesthesia (Ketamine 160 mg/kg and Xylasin 20 mg/kg). These muscles were chosen since FHL is composed predominantly of myosin heavy chain 2a/x (MHC IIa/x) with a small percentage of myosin heavy chain 2b (MHC IIb) [13,14], TA muscle is composed mainly of MHCIIb and soleus muscle is composed mainly of slow-fiber type I and IIa. Tibia bone length was measured for muscle weight normalization, since DEX treatment significantly reduces body weight. Muscles were immediately frozen and then stored at −80 °C for further analysis.

2.5. Western blot procedures

Samples of TA, FHL and SOL were homogenized in RIPA solution with 0.1% protease inhibitor cocktail and 1% of phenylmethylsulfonyl fluoride (PMSF), using a Polytron homogenizer as we previously published [4]. Samples were centrifuged at 10,000×*g* per 5 min and then the supernatant was collected and stored at −20 °C for future analysis. Bradford assays were used to determine the protein concentration (Bio-Rad Kit, Protein Assay Standart II, Hercules, CA, USA) as previously published [5–10]. Absorbance values were determined using a spectrophotometric plate reader (BMG labtech, Spectro Star Nano, Germany). Western blotting procedures were performed according to previously published studies [3,4]. In summary, 50–80 μg of protein were separated using 10% denaturing polyacrylamide gels with running buffer for 60 min. These gels were then transferred to nitrocellulose membranes using a transfer buffer for 90 min. The equal protein loading was confirmed using a 0.5% *Ponceau* S staining solution. Membranes were blocked with bovine serum albumin (BSA, 1.5%) in Tris-buffered saline with Tween (TBS-T) for 40 s. SNAP i.d.® 2.0 Protein Detection system (Millipore) was used to incubate the membranes with primary and secondary antibodies for 10 min as previously published by Macedo et al. [4]: primary antibodies in 3% BSA: atrogin-1 (Abcam #a74023, 1:1000), MuRF-1 (Santa Cruz (C-20) sc-27642, 1:1000) and myostatin/GDF-8 (Novus Biological (3E7) 1:500). Secondary antibodies were diluted in 1% BSA: atrogin-1 (Jackson Immuno Research #111-035-003, IgG anti-rabbit, 1:10,000), MuRF-1 (Jackson Immuno Research #705-035-003, anti-donkey, 1:10,000) and myostatin (Jackson Immuno Research #115-035-003, IgG anti-mouse, 1:5000) for 10 min. The antibody was detected by enhanced chemiluminescence (Super signal West Pico, Pierce®, Rockford, Illinois, USA) and the membranes were exposed to a radiographic film. The bands were analyzed using a computer program (Scion Image Corporation, version Beta 4.02, Frederick, Maryland, USA) and the values were expressed as percentage of control.

2.6. Statistics

The results are presented as mean ± standard error of mean. ANOVA Two-way with repeated measures was used (considering treatment and days as factors). The association between body weight and lean mass/muscle and protein level were assessed using Pearson correlation coefficient. Tukey post hoc test was used when necessary ($p < 0.05$). Software used: SigmaStat v.3.1.

3. Results

Fig. 1 illustrates DEXA scans of 2 representative animals (panel A, days 0, 5 and 10), lean mass (panel B) and body weight (BW, panel C) of rats through 10 days of DEX treatment, obtained by DEXA scan analysis. As shown in Fig. 1B, significant reductions in lean mass were observed since day 3 (−12%, −22%, −23% and −28%, for days 3, 5, 7 and 10, respectively, compared with their respective controls). All rats started the experimental protocol with similar BW (355 ± 11 g *vs* 346 ± 10 g, for C *vs* DEX, respectively). DEX treatment resulted in BW reductions from day-5 (−18%) up to days 7 (−21%) and 10 (−27%), when compared with respective control groups (Fig. 1, panel C), as suggested by DEXA images (panel A). We found a positive and highly significant correlation between lean mass and body weight of rats on days 5 ($r = 0.9466$) and 10 ($r = 0.9898$).

All groups presented similar values of fasting blood glucose before the experimental protocol. As shown in Table 1, DEX treatment significantly increased blood glucose since the first day of