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Dexamethasone Upregulates the Expression of the Nuclear Cofactor p300 and its Interaction With C/EBPβ in Cultured Myotubes

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Abstract Muscle wasting during sepsis and other catabolic conditions is, at least in part, mediated by glucocorticoids and is associated with upregulated transcription of multiple genes in the ubiquitin-proteasome proteolytic pathway. In addition to transcription factors, nuclear cofactors, including p300, regulate gene transcription. We tested the hypothesis that glucocorticoids upregulate the expression of p300 in muscle cells. Treatment of cultured L6 myotubes, a rat skeletal muscle cell line, with dexamethasone resulted in a dose- and time-dependent increase in p300 protein and mRNA levels. Surprisingly, the effect of dexamethasone on p300 levels was not inhibited by the glucocorticoid receptor (GR) antagonist RU38486 and RU38486 exerted an agonist effect on p300, increasing its expression. Co-immunoprecipitation showed that treatment of the myotubes with dexamethasone resulted in protein–protein interaction between p300 and C/EBPβ, but not C/EBPδ. The present results suggest that glucocorticoids upregulate the expression of p300 and its interaction with C/EBPβ in skeletal muscle. Increased expression and activity of p300 may be involved in the regulation of gene transcription in glucocorticoid-dependent muscle wasting. J. Cell. Biochem. 94: 1058–1067, 2005.

Key words: muscle wasting; glucocorticoids; transcription factors; nuclear cofactors; C/EBP; p300

Muscle wasting, mainly reflecting increased degradation of myofibrillar proteins, is a characteristic metabolic response to various catabolic conditions, including sepsis, severe injury, renal failure, AIDS, and cancer [Mitch and Goldberg, 1996; Hasselgren and Fischer, 2001; Price, 2003]. Although multifactorial, there is evidence that glucocorticoids are a predominant mediator of the increased muscle proteolysis seen in several of these conditions [Hasselgren, 1999]. Thus, sepsis- and injury-induced muscle breakdown can be prevented by treatment with the glucocorticoid receptor (GR) antagonist RU38486 [Hall-Angerås et al., 1991; Tiao et al., 1996]. In addition, treatment of rats [Auclair et al., 1997] or human subjects [Darmaun et al., 1988] with glucocorticoids results in increased muscle protein breakdown and dexamethasone-treated cultured muscle cells have been used as an in vitro model of muscle wasting [Wang et al., 1998; Du et al., 2000; Gomes et al., 2001].

There is evidence that the catabolic response in skeletal muscle is at least in part caused by increased expression and activity of the ubiquitin-proteasome proteolytic pathway [Mitch and Goldberg, 1996; Hasselgren and Fischer, 2001; Price, 2003]. The gene expression of several components of this pathway is increased in atrophying muscle, suggesting that transcriptional regulation plays an important role in muscle wasting. In recent studies from this laboratory, the expression and DNA binding activity of the "inflammatory" transcription factors NF-κB, AP-1, C/EBPβ, and C/EBPδ were increased in muscle from septic rats [Penner

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et al., 2001, 2002], lending further support to the concept that activation of gene transcription is an integral part of the catabolic response in skeletal muscle. Among transcription factors activated in atrophying muscle, C/EBPβ and δ are of particular interest because several genes in the ubiquitin-proteasome pathway have multiple potential C/EBP binding sites in their promoter regions [Penner et al., 2002].

In recent years, it has become increasingly clear that in addition to transcription factors, so called nuclear cofactors (coactivators or coregulators) are important for gene regulation [Janknecht and Hunter, 1996a]. One mechanism by which nuclear cofactors regulate gene transcription is histone acetylation resulting in disruption of chromatin and facilitated access of transcription factors to DNA binding sites [Polesskaya et al., 2001]. Another important mechanism by which nuclear cofactors influence transcriptional activity is protein–protein interactions with components of the basal transcription machinery and with various transcription factors [Janknecht and Hunter, 1996b]. Although, the mechanisms that regulate the functions of nuclear cofactors are not fully understood at present, the abundance of the cofactors may be a limiting factor, at least under certain circumstances [Vanden Berghe et al., 1999].

Among nuclear cofactors, p300 has received much attention due to its promiscuous interaction with a wide range of transcription factors, including C/EBP [Mink et al., 1997; Schwartz et al., 2003]. It is not known if the expression and activity of p300 are influenced by muscle wasting conditions and in particular, it is not known if muscle p300 is regulated by glucocorticoids. In the present study, we tested the hypothesis that glucocorticoids upregulate the expression of p300 in skeletal muscle and that this response is accompanied by an increased protein–protein interaction with C/EBPβ and δ. This was done by treating cultured myotubes with dexamethasone followed by measurement of the gene and protein expression of p300 and its interaction with C/EBP.

MATERIALS AND METHODS

Cell Culture

L6 rat skeletal muscle cells (American Type Culture Collection, Manassas, VA) were thawed and maintained by repeated subculturing at low density in 162-cm^2 culture flasks and were used between passages 2 and 8. Cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 μg/ml of streptomycin in 10% CO_2 atmosphere at 37°C. When cells reached approximately 80% confluence, they were removed by trypsinization and seeded into 150 mm culture dishes. The cells were grown in the presence of 10% FBS until they reached about 80% confluence at which time the medium was replaced with DMEM containing 2% FBS for induction of myotube differentiation. Cultures were used for experiments approximately 3 days later, when myotube formation was observed. Myotubes were treated with different concentrations of dexamethasone for various periods of time up to 24 h as indicated in Results. Untreated myotubes served as controls. In separate experiments, the GR antagonist RU38486 (Sigma, St. Louis, MO) was added to the culture medium at a concentration of 10 μM, either alone or in combination with 1 μM dexamethasone. After the different treatments, myotubes were harvested for determination of *p300* gene and protein expression and its interaction with the C/EBPβ and δ as described below.

Immunoprecipitation and Western Blotting

p300 was detected in nuclear or whole cell lysate extracts. For extraction of nuclear fractions, myotubes were harvested by scraping into ice-cold phosphate buffered saline (PBS) and pelleted by centrifugation at 3,800*g* for 5 min. Nuclei were isolated and nuclear fractions were extracted as described recently [Luo et al., 2003]. Whole cell lysates were prepared by lysing myotubes in three volumes of buffer (pH 7.4) containing protease inhibitor cocktail (Roche, Mannheim, Germany), 50 mM Tris-HCl, 0.3 M NaCl, 5 mM EDTA, and 1% Nonidet P-40. Protein concentrations in the nuclear and whole cell lysate extracts were determined with the BCA Protein Assay Reagent Kit (Pierce Biotechnology, Rockford, IL) using bovine serum albumin as standard. p300 in the nuclear and whole cell lysate fractions was immunoprecipitated using a rabbit polyclonal anti-p300 anitbody (N-15; Santa Cruz Biotechnology, Santa Cruz, CA). After 2 h of mixing at 4°C, 40 μl of packed Protein A/G PLUS-Agarose (Santa Cruz Biotechnology) was added and the samples were mixed at 4°C overnight. Immunoprecipitates were washed five times with 1 ml

of buffer containing 50 mM Tris-HC1, 0.15 M NaCl, 1 mM EDTA, and 1% Nonidet P-40 (pH 7.4) prior to resuspending the precipitates in sample buffer. Samples were then subjected to SDS–polyacrylamide gel electrophoresis using 4%–20% linear gradient Ready Gels (Bio-Rad Laboratories, Hercules, CA). The separated proteins were transferred to Immobilon PVDF Transfer Membranes (Millipore Corp., Bedford, MA) using a Trans-Blot SD semi-dry electrophoretic transfer cell (BioRad Laboratories) and p300 was detected by Western blotting using a mouse anti-human p300 monoclonal antibody (NM11; Pharmingen, San Diego, CA) as primary antibody and a goat anti-mouse IgG antibody (Promega, Madison, WI) as secondary antibody. Immunoreactive protein bands were detected using the Western Lightning™ kit for enhanced chemiluminescence detection (Perkin-Elmer Life Sciences, Boston, MA) and were exposed on Kodak X-Omat blue film (Eastman Kodak Co., Rochester, NY). Because p300 levels were determined by Western blotting of cell lysate aliquots that had been immunoprecipitated for p300 (rather than regular Western blotting of cell lysate total proteins), controls for equal loading of each lane could not be performed (and is typically not performed when immunoprecipitation has to be used for detection of the protein). Because only immunoprecipitated protein was added to each lane, no other proteins could be reliably detected on the gels. Aliquots from control and dexamethasone-treated myotube lysates containing identical amounts of total protein (as measured) were used for the immunoprecipitation. It was important to confirm that these aliquots were equivalent. To achieve that, we performed Western blotting of aliquots from control and dexamethasone-treated cell lysates used for p300 immunoprecipitation. For this Western blotting, an anti-α-tubulin antibody was used. This control, then, provided evidence that equal amounts of α-tubulin were present when a fixed amount of total protein (as measured) was sampled from the cell lysate for p300 immunoprecipitation. The controls also tested whether the changes in p300 levels were specific.

Co-Immunoprecipitation

Co-immunoprecipitation was used to detect protein–protein interaction between p300 and C/EBPβ and δ. Immunoprecipitation of p300 in whole cell extracts was performed as described above using the N-15 anti-p300 antibody. Proteins in the immunoprecipitate were separated on 10% Ready Gels (Bio-Rad Laboratories) and after transfer to Immobilon PVDF Transfer Membranes (Millipore Corp.), C/EBPβ and δ were detected by Western blotting using a mouse anti-human C/EBPβ and a rabbit anti-rat C/EBPδ antibody (Santa Cruz Biotechnology) as primary antibodies and goat anti-mouse and anti-rabbit IgG antibodies (Promega) as secondary antibodies. Thus, in this experiment, Western blotting detected C/EBPβ and δ that was bound to p300.

Real-Time PCR

p300 mRNA levels were determined by real-time PCR performed as described in detail recently [Okuno et al., 2002]. Total RNA was extracted by the acid-guanidinium thiocyanate–phenol–chloroform method [Chomczynski and Sacchi, 1987] using Tri Reagent (MRC, Inc., Cincinnati, OH). Multiplex RT-PCR for quantitation of rat p300 expression with amplification of 18S RNA as endogenous control, TaqMan analysis, and subsequent calculations were performed with an ABI Prism® 7700 Sequence Detection System (Perkin-Elmer, Foster City, CA). This system detects the increase in fluorescent signal released from an internal fluorogenic probe as the PCR proceeds. For each sample, 100 ng of total RNA was subjected to real-time PCR according to the protocol provided by the manufacturer of the TaqMan One Step PCR Master Mix Reagents Kit (Part #4309169, ABI, Foster City, CA) The sequences of the forward, reverse, and double-labeled oligonucleotides were as follows, respectively: 5′-GCCAAACATGCAGTACCCAA-3′, 5′-CCCTGCTGTAGTGGCTCAGTC-3′, and FAM-AGGCATGGGCAATGCTGGCAGTT-TAMRA. Amplification of 18S RNA was performed in the same reaction tubes as an internal standard with an alternatively labeled probe (VIC labeled probe) to distinguish its product from that derived from p300 RNA. Experiments were performed in triplicate for each standard and L6 cell sample. The p300 level in control myotubes was arbitrarily set to 1.0 and the levels in dexamethasone-treated myotubes were expressed as fold increase. The p300 mRNA levels were normalized to 18S mRNA. In addition, mRNA levels for GAPDH were determined to test for the specificity of changes in p300 mRNA levels.

Statistics

Results are given as means ± SD. Experiments were repeated at least three times to provide evidence of reproducibility.

RESULTS

In previous studies, we found that treatment of cultured L6 myotubes with dexamethasone resulted in increased ubiquitin-proteasome-dependent protein degradation with a maximal effect seen after treatment with 1 μM dexamethasone for 6 h [Wang et al., 1998]. Here, we found that the same treatment resulted in increased cellular levels of p300 (Fig. 1). Because in initial experiments, identical results with regards to the effect of dexamethasone were observed when p300 was examined in whole cell lysates or nuclear fractions (data not shown), we used whole cell lysates in subsequent experiments. Further analysis of the effect of dexamethasone on myotube p300 levels revealed that the changes in p300 levels were time- and dose-dependent with a maximal effect seen after 8–16 h in cells treated with 0.5–1 μM dexamethasone (Figs. 2 and 3). The myotubes were sensitive to the effects of dexamethasone with an increase in p300 levels seen in cells treated with 0.05 μM dexamethasone. When the myotubes were treated with 1 μM dexamethasone, p300 levels were increased already after 2 h. Thus, p300 levels were upregulated earlier and at lower dexamethasone concentrations than we observed previously for protein breakdown rates in dexamethasone-treated L6 myotubes [Wang et al., 1998]. Because in the present study, we wanted to examine the effect of dexamethasone on p300 levels in a model that reflected the increase noticed previously in protein degradation [Wang et al., 1998], in most subsequent experiments in the present study, myotubes were treated with 1 μM for 8 h.

Increased myotube levels of p300 may reflect inhibited degradation or upregulated synthesis

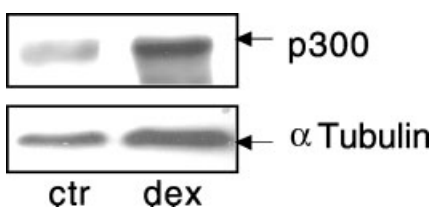

Fig. 1. p300 levels in cultured L6 myotubes treated for 6 h with 1 μM dexamethasone (Dex). Untreated L6 myotubes served as controls (ctr). p300 levels were determined by immunoprecipitation as described in Materials and Methods. Almost identical results were observed in three repeated experiments. α-Tubulin levels were determined by Western blotting of cell lysates from control and Dex-treated myotubes using identical aliquots that were used for immunoprecipitation of p300.

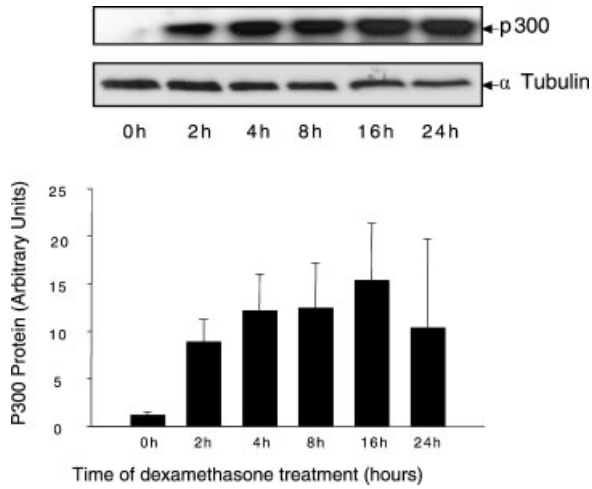

Fig. 2. p300 levels in cultured L6 myotubes treated for different periods of time with 1 μM Dex. A representative immunoblot is shown in the **upper panel**. α-Tubulin levels were determined by Western blotting of cell lysates from control and Dex-treated myotubes using identical aliquots that were used for immunoprecipitation of p300. Quantification of multiple p300 immunoblots by densitometry is shown in the **lower panel** in which results are given as means ± SD with n = 3 for each time point.

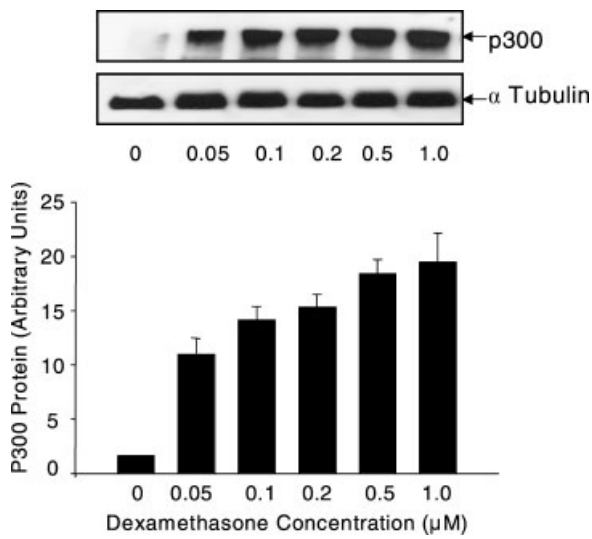

Fig. 3. p300 levels in L6 myotubes treated with different concentrations of Dex for 8 h. A representative immunoblot is shown in the **upper panel**. α-Tubulin levels were determined by Western blotting of cell lysates from control and Dex-treated myotubes using identical aliquots that were used for immunoprecipitation of p300. Quantification by densitometry of p300 immunoblots is shown in the **lower panel**. Results are means ± SD with n = 3 for each data point.

of the protein or a combination of these changes. In order to test whether dexamethasone influenced the stability of p300, myotubes were first treated with 1 μM dexamethasone for 2 h (in order to increase p300 levels) whereafter the cells were washed and incubated for an additional 24 h in a medium containing 10 μg/ml of the protein synthesis inhibitor cycloheximide in the absence or presence of 1 μM dexamethasone. Results from that experiment showed that the decline in p300 levels was not affected by dexamethasone, suggesting that dexamethasone did not influence the degradation of p300 (Fig. 4).

In order to test the role of protein synthesis in dexamethasone-induced increase in myotube p300 levels, cells were treated with 1 μM dexamethasone in the absence or presence of 10 μg/ml of cycloheximide. Cycloheximide inhibited the increase in p300 levels observed after treatment with dexamethasone suggesting that the increased p300 levels reflected increased synthesis of the protein (Fig. 5). In the same experiment, actinomycin D completely abolished the dexamethasone-induced expression of p300, suggesting that the increased p300 production was regulated at the transcriptional level. The potential role of transcriptional upregulation was further supported by increased p300 mRNA levels in the dexamethasone-treated myotubes (Fig. 6) although this increase

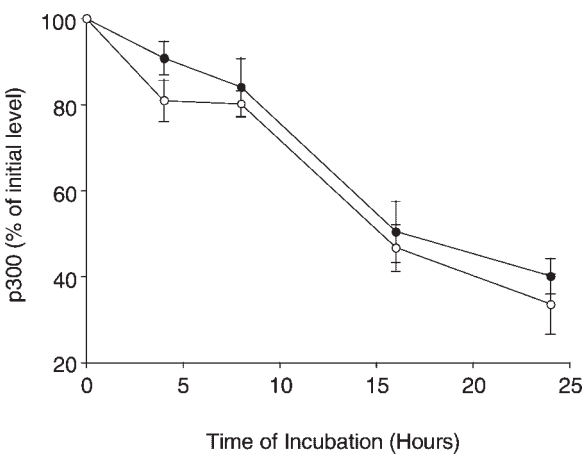

Fig. 4. p300 levels in L6 myotubes that had first been treated with Dex for 2 h in order to increase p300 levels and that were then incubated up to 24 h in the presence of 10 μg/ml of cycloheximide. Myotubes were incubated in the absence (open circle) or presence (filled circles) of 1 μM Dex. p300 levels were quantified by densitometry. Results are means ± SD with n = 3 for each data point.

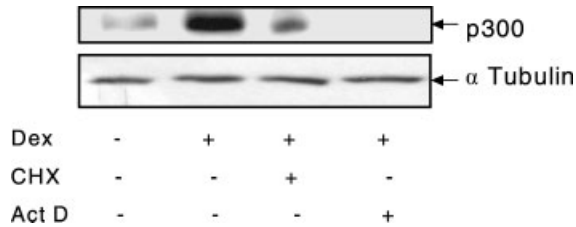

Fig. 5. p300 levels in L6 myotubes treated for 8 h with 1 μM Dex in the absence or presence of 10 μg/ml of cycloheximide (Chx) or 5 μM actinomycin-D (Act D). Similar results were observed in three repeated experiments.

was relatively modest (approximately 1.5-fold). The mRNA levels shown in Figure 6 were normalized to 18S mRNA. In additional experiments, we found that GAPDH mRNA levels (also normalized to 18S mRNA) were not affected by dexamethasone treatment (data not shown), suggesting that the increase in p300 mRNA levels did not reflect a generalized, nonspecific increase in gene expression. The more pronounced increase in p300 protein than mRNA levels (compare Figs. 2 and 6) suggests that p300 protein expression was influenced by both transcriptional and post-transcriptional regulation, possibly increased translational efficiency.

In some [Polesskaya et al., 2001], but not all [Puri et al., 1997], previous studies, p300 was differentially regulated in myoblasts and myotubes. It is not known if the regulation of p300 by glucocorticoids is different in myoblasts and differentiated myotubes. We, therefore, compared

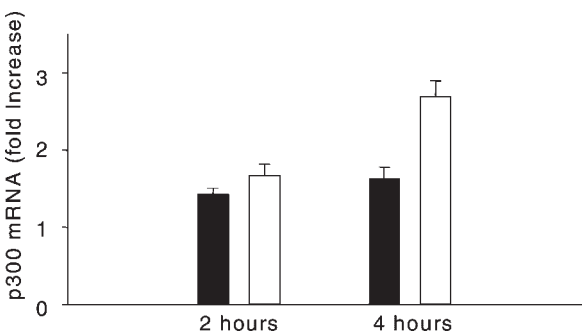

Fig. 6. p300 mRNA levels determined by real-time PCR in L6 myotubes treated for 2 or 4 h with 1 μM Dex in the absence (filled bars) or presence (open bars) of 10 μg/ml of cycloheximide. Results are given as fold increase compared to mRNA levels in untreated myotubes. Results are means ± SD with n = 3 for each group.

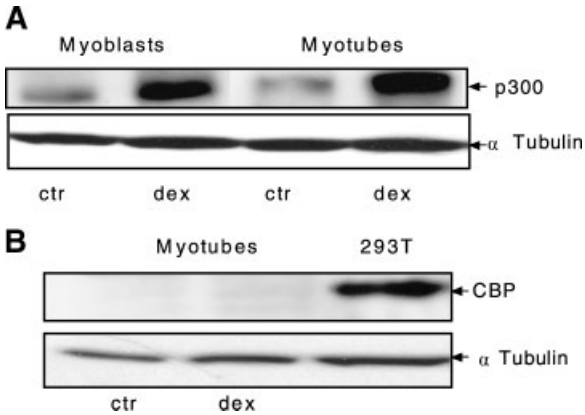

Fig. 7. **A**: p300 levels in L6 myoblasts and myotubes treated for 8 h with 1 μM dexamaethasone (dex). Control myotubes (ctr) were cultured in the absence of Dex. **B**: CBP levels in myotubes cultured for 8 h in the absence (ctr) or presence of 1 μM Dex. The third lane represents CBP levels in 293T cells transfected with a CMV-HA-CBP expression plasmid. Similar results were observed in three repeated experiments. α-Tubulin levels were determined by Western blotting of cell lysates from control and Dex-treated cells using identical aliquots that were used for immunoprecipitation of p300.

the effect of dexamethasone on p300 levels in undifferentiated myocytes and myotubes and found that the glucocorticoid-induced expression of p300 was independent of myocyte differentiation (Fig. 7A).

p300 is closely related to another nuclear cofactor, the CREB-binding protein CBP [Shikami et al., 1997]. In fact, because of the similarities, these proteins are frequently referred to as p300/CBP. They are commonly, but not always, expressed in the same cell and regulated by various treatments in a parallel fashion. We next tested whether CBP was expressed in L6 myotubes and regulated by dexamethasone. We could not detect CBP in untreated or dexamethasone-treated myotubes (Fig. 7B). To make certain that this finding did not reflect a defective CBP antibody, the same antibody was used in human kidney epithelial 293T cells transfected with a CMV-HA-CBP expression plasmid (kindly provided by Dr. Hong Wang, Department of Cardiology, Beth Israel Deaconess Medical Center, Harvard Medical School). In this experiment, Western blotting yielded a strong signal at the expected molecular weight for CBP (Fig. 7B). Taken together, these results suggest that CBP is not expressed at the protein level in normal or dexamethasone-treated L6 myotubes.

In order to further characterize the regulation of p300 expression by dexamethasone, we tested whether cycloheximide would influence the dexamethasone-induced increase in p300 mRNA levels. Results from that experiment showed that cycloheximide did not prevent the increase in p300 mRNA levels seen in dexamethasone-treated myotubes (Fig. 6). This observation suggests that glucocorticoids regulate the *p300* gene through a primary mechanism [Falkenstein et al., 2000].

Although most effects of glucocorticoids are secondary to activation of the GR, recent studies suggest that glucocorticoids may induce some metabolic changes independent of GR binding [Chen and Qiu, 1999; Falkenstein et al., 2000; Qiu et al., 2001]. In order to test the role of GR binding in dexamethasone-induced increase in p300 levels, we examined the influence of RU38486 on p300 levels in dexamethasone-treated myotubes. Surprisingly, RU38486, at a ten molar excess of dexamethasone, did not prevent the dexamethasone-induced increase in p300 levels (Fig. 8). In the same experiment, RU38486 exhibited agonist activity and by itself induced increased p300 levels.

One important mechanism by which nuclear cofactors regulate gene transcription is protein–protein interaction with various transcription factors. Recent studies from our laboratory suggest that the transcription factors C/EBPβ and δ are activated in atrophying muscle during sepsis and that this effect of sepsis may at least in part be regulated by glucocorticoids [Penner et al., 2002]. It is not known if glucocorticoids increase the interaction between p300 and C/EBPβ or δ in skeletal muscle. We, therefore, next used co-immunoprecipitation to test whether treatment of myotubes with dexamethasone resulted in protein–protein interaction between p300 and the β and δ isoforms of the C/EBP family. Results from that experiment provided evidence that dexamethasone resulted

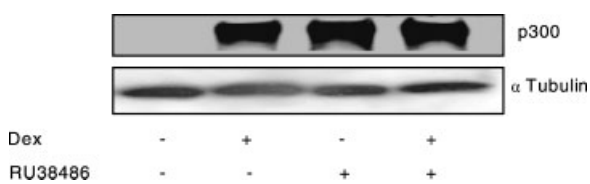

Fig. 8. p300 levels in L6 myotubes treated for 8 h with 1 μM Dex and/or 10 μM RU38486 as indicated below the blot. Identical results were observed in three repeated experiments.

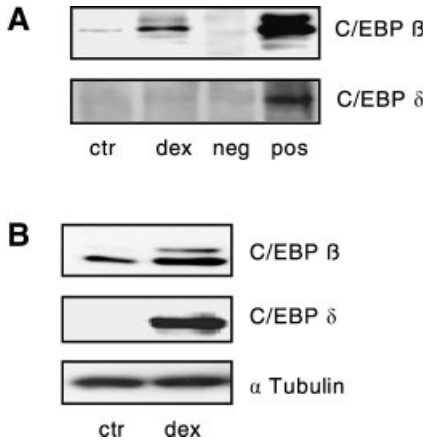

Fig. 9. **A**: Interaction between p300 and C/EBPβ or δ in untreated (ctr) L6 myotubes and in L6 myotubes that were treated for 8 h with 1 μM Dex. The protein–protein interaction between p300 and C/EBPβ or δ was detected by co-immunoprecipitation using an anti-p300 antibody for pull down and an anti-C/EBPβ or δ antibody for Western blotting as described in Materials and Methods. Neg, negative control in which non-specific IgG was used instead of anti-p300 antibody. Pos, positive control in which cell lysate extract (25 μg protein) from L6 myotubes treated with 1 μM Dex for 8 h was added to the gel. **B**: Western blotting of cell lysates from control myotubes (ctr) and myotubes treated with 1 μM Dex for 8 h.

in protein–protein interaction between p300 and C/EBPβ (Fig. 9A). In contrast, there was no evidence of protein–protein interaction between p300 and C/EBPδ. In additional experiments, treatment of myotubes with dexamethasone resulted in a pronounced increase in both C/EBPβ and δ protein levels determined by Western blotting and using the same antibodies as used in the co-immunoprecipitation experiments (Fig. 9B). Thus, the lack of evidence for interaction between p300 and C/EBPδ noticed here did not reflect a defective C/EBPδ antibody.

DISCUSSION

In the present study, treatment of cultured L6 myotubes with dexamethasone resulted in upregulated p300 mRNA and protein levels and increased protein–protein interaction between p300 and C/EBPβ. Because in other studies, glucocorticoids increased muscle protein breakdown [Hall-Angerås et al., 1991; Tiao et al., 1996; Hasselgren, 1999], activated the transcription factor C/EBPβ [Penner et al., 2002], and upregulated the expression of various genes in the ubiquitin-proteasome proteolytic pathway [Wang et al., 1998; Du et al., 2000; Fischer et al., 2000; Gomes et al., 2001], it is possible that increased expression and activity of p300 plays a role in glucocorticoid-regulated muscle wasting. Further studies are needed, however, to more definitively test the potential link between increased p300 expression and activity and protein degradation in dexamethasone-treated myotubes.

To our knowledge, the present study is the first report of glucocorticoid-induced upregulation of p300 expression in skeletal muscle or any other cell type. In other studies, evidence was found that increased p300 levels can regulate glucocorticoid-induced gene transcription. For example, in a recent report by Li et al. [2002], dexamethasone-induced transcription of the mouse mammary tumor virus (*MMTV*) gene was potentiated several-fold in cells in which the p300 levels were increased by transfection with a p300 expression plasmid. Although, the influence of glucocorticoids on p300 expression was not specifically studied by Li et al. [2002], results in some of their experiments suggested that p300 levels were not altered by treatment of HeLa cells for up to 20 h with 0.1 μM dexamethasone, a concentration of dexamethasone that increased p300 levels substantially in L6 myotubes in the present study. Thus, it is possible that the regulation of p300 expression by glucocorticoids is cell specific.

p300 can influence gene transcription through multiple mechanisms, including histone acetyl transferase (HAT) activity and interaction with various transcription factors. Interestingly, when HeLa cells were treated with dexamethasone, increased MMTV gene transcription was the result of p300-GR association during the first 2–4 h of treatment but reflected increased HAT activity after more prolonged dexamethasone treatment [Li et al., 2002]. It is not known from the present study if gene transcription was influenced by the increased p300 levels in the dexamethasone-treated myotubes. We found, however, that the interaction with at least one transcription factor that has been found to be associated with increased expression of genes in the ubiquitin-proteasome proteolytic pathway, C/EBPβ, was increased in myotubes expressing high levels of p300.

Results in the present study suggest that dexamethasone-induced increase in myotube p300 levels reflected increased synthesis, rather than reduced degradation, of the protein. Recent studies suggest that changes in p300

degradation may be important for the regulation of p300 levels in other conditions. For example, treatment of cultured cardiomyocytes with doxorubicin or of HeLa cells with sodium butyrate reduced cellular p300 levels by activating proteasome-dependent degradation of the protein without altering p300 synthesis [Poizat et al., 2000]. Interestingly, proteasome-dependent degradation regulated p300 levels in sodium butyrate-treated HeLa cells without influencing basal p300 levels in the same cells [Li et al., 2002]. Thus, the role of altered synthesis versus degradation for changes in p300 levels may vary with cell type and treatment.

One important function of nuclear cofactors is to influence gene transcription by binding to various transcription factors. C/EBP was added only recently to the growing list of transcription factors with which p300 interacts [Mink et al., 1997; Schwartz et al., 2003]. Results in the present study suggest that p300 interacts selectively with individual C/EBP isoforms in dexamethasone-treated myotubes. In contrast, p300 interacted with several members of the C/EBP family of transcription factors, including C/EBPα, β, and δ, in cultured QT6 cells [Schwartz et al., 2003]. Although, the mechanisms by which the interaction between p300 and transcription factors influence gene transcription are not fully understood at present, there is evidence that phosphorylation and acetylation of transcription factors induced by p300 binding may be involved [Shikami et al., 1997]. Interestingly, results in a recent study showed that the recruitment of p300 by C/EBPβ induced a substantial phosphorylation and activation of the cofactor [Schwartz et al., 2003]. Thus, the protein–protein interaction between p300 and C/EBPβ, and probably other transcription factors as well, results in complex changes of both the cofactor and transcription factor that may be important for the regulation of gene transcription.

An interesting observation in the present study was that the dexamethasone-induced expression of p300 was not inhibited by RU38486. Although most effects of glucocorticoids are typically the result of binding to and activation of the GR, there is recent evidence that glucocorticoids can also influence cell biology by GR-independent mechanisms [Chen and Qiu, 1999; Qiu et al., 2001]. Results from experiments in other cell types suggest that GR-independent responses to glucocorticoid treatment may at least in part be mediated by membrane-associated receptors, activating different intracellular signaling pathways [Guo et al., 1995; Lackner et al., 1998].

In addition to being unable to prevent the effect of dexamethasone on p300 levels, RU38486 had an agonist effect on myotube p300 levels. A similar correlation between inability to block glucocorticoid-induced metabolic changes and an agonist effect of RU38486 was reported in other cell types as well and may reflect the involvement of various signaling pathways and co-activators/repressors in glucocorticoid agonist activity [Beck et al., 1993; Liu et al., 2002; Schulz et al., 2002; Zhang et al., 2003]. In recent experiments, we found that dexamethasone-induced upregulation of C/EBPβ expression in L6 myotubes was not blocked by RU38486 whereas the drug effectively inhibited the expression of C/EBPδ (unpublished observations). Whether that observation is related to the present finding that dexamethasone resulted in interaction between p300 and C/EBPβ but not C/EBPδ remains to be determined.

Although, the present study is the first report of glucocorticoid-induced regulation of p300 expression in muscle cells, several previous reports provided evidence that p300 is expressed in skeletal muscle [Yuan et al., 1996; Puri et al., 1997; McKinsey et al., 2001; Polesskaya et al., 2001]. In those studies, p300 was found to interact with multiple muscle-specific transcription factors, including MyoD [Yuan et al., 1996; Puri et al., 1997], and to play an important role in muscle differentiation [McKinsey et al., 2001; Polesskaya et al., 2001]. In previous studies, both p300 and the closely related protein CBP were expressed and played almost identical roles with regards to cell differentiation in cultured C2C12 cells, a mouse skeletal muscle cell line [Polesskaya et al., 2001]. Surprisingly, CBP protein was not expressed in untreated or dexamethasone-treated L6 myotubes in the present study. The reason for this finding is unclear but may be related to different expression of CBP in mouse and rat muscle.

In summary, the present study reports for the first time that dexamethasone upregulates the expression of p300 and its interaction with C/EBPβ in cultured muscle cells. It will be important in future studies to determine whether

these changes are involved in glucocorticoid-induced gene transcription and muscle wasting.

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