Sepsis induces the transcription of the glucocorticoid receptor in skeletal muscle cells

Xiaoyan SUN*,†, Joshua M. V. MAMMEN* and Xintian TIAN‡

*Department of Surgery, The University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267, U.S.A., †Shriners Hospitals for Children, 3229 Burnet Avenue, Cincinnati, OH 45229, U.S.A., and ‡Department of Molecular and Cellular Physiology, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267, U.S.A.

Abstract

Evidence from a recent study indicates that glucocorticoids (GCs) mediate skeletal muscle proteolysis during sepsis via the GC receptor (GR) pathway. Attempts to identify the mechanisms regulating GR gene expression in skeletal muscle during sepsis have been hampered by the lack of an appropriate in vitro model system that can mimic in vivo septic conditions. In the present study, we report that GR gene transcription in L6 myocytes in vitro is up-regulated by treatment with sera from septic rats in a manner similar to that measured in septic rats in vivo. Sera from septic rats were collected from animals in which sepsis was induced by caecal ligation and puncture and from control rats that were sham-operated. Finally, by treating L6 myotubes with the GR antagonist RU 38486, thereby preventing sepsis-induced GR transcription, we confirmed that the possible septic effect on the GR was due to increased GCs. L6 myocytes treated with sera from septic rats might therefore be useful as an experimental model for identifying the molecular mechanisms by which the GR regulates muscle cachexia during sepsis. Furthermore, RU 38486 inhibited the sepsis-induced increase in total and myofibrillar energy-dependent protein breakdown rates in incubated extensor digitorum longus muscles from septic and sham-operated rats, as measured by release of tyrosine and 3-methylhistidine respectively. Our results demonstrate for the first time that sepsis induces GR transcription in skeletal muscle, and supports the hypothesis that the GC-induced proteolysis under sepsis is partially a consequence of GR activation.

Introduction

During sepsis, catabolic changes are induced by mediators such as hormones, cytokines, transcriptional factors or lipid metabolites in many organ systems [1]. Glucocorticoids (GCs) play a major role in the regulation of many intracellular metabolic and immunological events that characterize the systemic response to infection and inflammation [1–3]. One of the most prominent metabolic consequences of sepsis is muscle catabolism, which is characterized by myofibrillar protein breakdown [2,4] caused by an increase in non-lysosomal energy-dependent proteolysis [5]. The introduction of the GC receptor (GR) antagonist RU 38486 (mifepristone) [6] has facilitated the evaluation of the role of circulating GCs in this process. When administered to septic rats, RU 38486 blocks the increase in ubiquitin mRNA and free ubiquitin levels and inhibits energy-dependent myofibrillar proteolysis [7,8]. However, how GCs influence myocellular energy production is largely unknown.

We have reported previously [9] that sepsis induces significant changes in GR-binding activity and GR expression in the bioenergetics of resting skeletal muscles. Prominent among these changes is an increase in GCs...
in plasma of septic rats, which may mediate GR up-regulation during sepsis. GR belongs to the superfamily of ligand-modulated transcription factors that can regulate gene transcription by activation (positive) or repression (negative) [10,11] depending on the DNA sequences in the target promoters (termed GC-response elements (GREs), a consensus cis-acting DNA sequence AGAA-CANNNTTGTC (GRE) in the 5’ upstream-controlling region of the genes) to which the receptor binds [12]. The number of GREs and their position relative to the transcriptional start site may be an important determinant of the magnitude of the transcriptional response to steroids [13–15]. Thus an increased number of GREs and proximity to the TATA box increases steroid inducibility of a gene [13–15]. The aim of the present study was to evaluate the role of GCs in the regulation of these GR changes in muscle under septic conditions. First, we tested the hypothesis that the central effects of sepsis, which lead to an increased transcriptional regulation of the GR, are due to the increasing circulation of GCs inducing a GR-dependent promoter. Moreover, sepsis elicits a GR response in skeletal muscles in vivo [9]. To this end, we used two cell culture models of skeletal muscles to test for the transactivation of the GR by dexamethasone (DEX) under septic conditions. Secondly, we attempted to clarify whether sepsis indeed activates the GR, since the answer to this question has far-reaching implications for both steroid receptor research and its clinical applications. Therefore we addressed the remaining arguments (cell type, treatment condition or promoter specificity) that could potentially explain the discrepancy described above, i.e. we included rat L6 and mouse C2C12 myocytes in our experiments; tested sera from septic and endotoxaemic rats, the effect of cortisol (CORT) and DEX treatments; and used mouse mammary tumour virus (MMTV) and GRE reporter genes.

**MATERIALS AND METHODS**

**Experimental animals**

Sepsis was induced in male Sprague–Dawley rats (40–60 g body weight) by caecal ligation and puncture (CLP), whereas control rats underwent sham-operation, i.e. laparotomy and manipulation, but no ligation or puncture of the caecum, as described previously [9]. All rats were resuscitated with 10 ml/100 g of body weight of normal saline administered subcutaneously on the back at the time of surgery to prevent hypovolaemia and septic shock. Rats had free access to drinking water after the surgical procedures, but food was withheld in order to avoid the influence of any differences in food intake between the groups of rats on GR transcription.

The septic model has been applied to a number of previous studies to investigate sepsis-related metabolic changes [8,9,16–19], which is clinically relevant, because it results in hyperdynamic and hypermetabolic sepsis at 16–18 h after CLP and resembles the situation in many surgical patients with sepsis caused by intra-abdominal abscess and devitalized tissue. In order to test the effects of GR on muscle proteolysis, groups of rats were injected subcutaneously with 10 mg/kg of DEX or a corresponding volume of PBS (vehicle) 2 h before CLP or sham-operation. RU 38486 was administered by gavage or a corresponding volume (0.5 ml/100 g of body weight) of vehicle 2 h before CLP or sham-operation either with DEX treatment or PBS as described previously [9].

In additional experiments, endotoxaemia was induced in rats by the subcutaneous injection of 12.5 mg/kg of endotoxin (Escherichia coli endotoxin O111:B4; Calbiochem, La Jolla, CA, U.S.A.). Control rats were injected with a corresponding volume (0.5 ml) of sterile saline. All reagents and chemicals were purchased from Sigma (St. Louis, MO, U.S.A.) unless indicated.

The animal work was undertaken as required by the Animal Care Committees at the University of Cincinnati, and the experimental procedures were carried out in accordance with the United States National Institutes of Health guidelines.

**Plasma sera**

At different time points after CLP or sham-operation (2, 4, 6, 8 and 16 h), and at 16 h after injection of endotoxin or saline, rat blood was collected by cardiac puncture to treat cells as described previously [9,20].

**Myocytes culture**

L6 (a rat skeletal muscle cell line) and C2C12 (a mouse skeletal muscle cell line) myocytes were cultured as described previously [21,22]. Briefly, cells were thawed and maintained by repeated subculturing at low density on 10-cm diameter culture dishes and were used between passages 2 and 8. Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY, U.S.A.) supplemented with 10 % (v/v) fetal calf serum (FCS), 100 units/ml of penicillin, 100 µg/ml streptomycin, 44 mM NaHCO3 and 100 µg/ml sodium pyruvate in a humidified atmosphere of 10 % CO2 and 90 % air at 37°C. L6 myoblasts were plated at 60 % confluency in 100-mm dishes for transfection.

**Cell transfection and induction**

Cells were seeded into plastic dishes (60-mm diameter) 2 days before transfection in DMEM containing 10 % (v/v) charcoal-stripped steroid-free FCS. Dextran T-70 (Amersham Biosciences, Piscataway, NJ, U.S.A.) was used for charcoal-stripping of FCS. The putative residual cortisol concentration was determined by HPLC and found to be below the detection limit, i.e. below 0.036 nM in DMEM containing the steroid-free FCS (results not shown). In addition, the luciferase activity of L6 myocytes incubated in the absence or presence of the GR
antagonist RU 38486 (1 μM) showed no difference in the reporter assay described below, whereas RU 38486 efficiently suppressed cortisol induction of the GRE-dependent reporter gene (results not shown). Thus the activity of putative residual steroids is negligible.

When cells reached 50% confluence, the medium was renewed and calcium phosphate-precipitated plasmid DNA was distributed over the cells. The precipitate was prepared 30 min before addition to the cells and consisted of 2.0 µg of steroid-responsive luciferase reporter plasmid [pMMTV–Luc (Clontech, Palo Alto, CA, U.S.A.) or ptk-(GRE)2–Luc (Gibco)], 2.0 µg of simian virus 40 promoter-driven β-galactosidase expression vector pCMV-SPORT–βgal (Gibco) and, where indicated, 1.0 µg of pCMV:GR, which expresses rat GR from the cytomegalovirus promoter of the vector pRSVGR (provided by Dr K. R. Yamamoto, Department of Cellular and Molecular Pharmacology, University of California at San Francisco, San Francisco, CA, U.S.A.) [23]. Plasmid DNA was diluted into 200 µl of Hanks balanced salt buffer [137 mM NaCl, 5 mM KCl, 0.7 mM Na2HPO4 (H2O), 6 mM d-glucose and 20 mM Hepes (pH 7.1)], to which 10 µl of 2.5 M CaCl2 was slowly added. Cells were cultured for an additional 5–6 h, the medium was removed, 2 ml of PBS supplemented with 15% (v/v) glycerol was added for 2 min, and cells were washed twice with PBS. Cells were cultured for 16 h in fresh DMEM containing 10% (v/v) steroid-free FCS and supplemented with different sera (sera from sham-operated, septic, saline-treated or endotoxaemic rats), DEX, CORT and RU 38486 in the combinations and concentrations as indicated. DEX, CORT and RU 38486 were dissolved in ethanol, and sera from sham-operated, septic, vehicle-treated and endotoxaemic rats were dissolved in DMSO. Additional solvent was added to each cell dish, so that the total concentration of solvent was the same throughout each experiment.

Luciferase and β-galactosidase assay
Medium was removed and 1 ml of lysis buffer [0.1 M KH2PO4 (pH 7.8) and 1 mM dithiothreitol] was added to each plate (60-mm diameter). Cells were scraped from the plate, transferred to a 1.5 ml reaction tube, and subjected to three freeze (3 min at 80 °C) and thaw (3 min at 37 °C) cycles. After centrifugation (13 793 g, room temperature, 4 min; Biofuge, Heraeus, Muskegon, MI, U.S.A.), 50 µl of each supernatant [corresponding to approx. (1–2) × 10⁶ cells] was transferred to a 96-well plate. Subsequently, 150 µl of solution containing 33 mM KH2PO4 (pH 7.8), 1.7 mM ATP, 3.3 mM MgCl2 and 13 mM Luciferin™ (Gibco–Brl) was added to each sample by an automatic luminometer (Luminat LB 96; Wallac GmbH, Freiburg, Germany), and light emission was measured for 10 s. To correct for variations in transfection efficiency, values of the luciferase assay were normalized with β-galactosidase activities that were measured as follows: 50 μl of cell extract was added to 100 μl of galactosidase buffer [60 mM Na2HPO4, 40 mM NaH2PO4, 10 mM KCl, 1 mM MgCl2, 50 mM 2-mercaptoethanol (pH 7.4)] on a 96-well plate. Then, 20 µl of 2 mg/ml o-nitrophenyl β-d-galactopyranoside was added and the reaction was incubated at 37 °C. After 10–30 min, absorption was measured at 405 nm in a spectrophotometer (MR5000; Dynatech, Chantilly, VA, U.S.A.).

Total and myofibrillar protein breakdown in incubated muscles
Total and myofibrillar protein breakdown in incubated muscles were measured as described previously [8]. Briefly, 16 h after CLP or sham operation and injection with DEX or RU 38486, rats were anaesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) and the extensor digitorum longus muscles were gently dissected and excised with intact tendons. The muscles were mounted on stainless steel supports at resting length and immediately placed in 3 ml of oxygenated (O2/CO2, 19:1) Krebs–Henseleit bicarbonate buffer [25 mM NaHCO3, 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM NaH2PO4 and 1.2 mM CaCl2 (pH 7.4)] with 5 mM glucose at 37 °C in a shaking water bath for 30 min (except in the experiments in which muscles were energy-depleted when preincubation was carried out for 90 min, see below). After preincubation, one muscle was homogenized in 0.4 M perchloric acid for determination of tissue levels of free tyrosine and 3-methylhistidine (3-MH) by HPLC. The contralateral muscle was incubated for 2 h in fresh medium of the same composition as described above with the addition of 0.5 mM cycloheximide to prevent reincorporation of amino acids released during proteolysis. After incubation, muscle and medium concentrations of free tyrosine and 3-MH were measured. Total and myofibrillar protein breakdown rates were determined from the net release as nmols of tyrosine/g of wet weight per 2 h and nmols of 3-MH/g of wet weight per 2 h respectively.

Plasma corticosterone levels
At different time points up to 16 h after CLP or sham operation, or 16 h after endotoxin or saline injection, rat blood was collected by cardiac puncture to determine corticosterone levels. Plasma corticosterone concentrations were measured using commercially available RIA kits (ICN Biochemicals Inc., Costa Mesa, CA, U.S.A.) as described previously [9].

Statistical analysis
Results are presented as means ± S.E.M. Student’s t test or ANOVA was used for statistical analysis as appropriate.
Figure 1 Effect of septic sera on transcription of GR in L6 myocytes

(A) L6 myocytes were transfected with a GR-dependent reporter plasmid (pMMTV–Luc) and a β-galactosidase-expressing control plasmid (pCMV·SPORT–βgal). After transfection, cells were cultured with sera obtained at 2, 4, 6, 8 and 16 h following sham-operation (●) or CLP (○). Luciferase activities were corrected using the results from the β-galactosidase assay and are presented as fold induction with untreated cells as a reference. (B) A rat GR-expressing plasmid (pCMV:GR) was cotransfected with pMMTV–Luc and then treated with sera obtained at 6 h from sham-operated or septic (SEP) rats under the same conditions as in (A). Results are means ± S.E.M. of three independent experiments. *P < 0.01 among all groups by ANOVA.

RESULTS

Sepsis-induced GR gene transcription

To test the hypothesis that GR activation by sepsis in skeletal muscles is through transcription, we initially used L6 myocytes to characterize the effect of sepsis on the GR. This cell line is derived from rat skeletal muscle [24] and is richly endowed with GR [25]. Thus L6 myocytes can be considered as an ideal model for studying GC-mediated proteolysis in skeletal muscles. We tested the activation of the steroid-dependent MMTV promoter, which contains several GREs [26], in transient transfection assays by adding sera obtained at various time points after the induction of sepsis. Figure 1(A) shows that cells incubated with sera obtained from septic rats obtained between 2–16 h after CLP activated the promoter. We also assayed MMTV promoter activity in L6 myocytes overexpressing rat GR. Again, sera from septic rats stimulated an approx. 50-fold increase in transcription from the MMTV promoter (Figure 1B). Since cell-type specificity has been reported for GC-autoregulated GR activity [9,27–29], it is therefore possible that sepsis activates the GR only in rat L6 myocytes. To address this issue, we also used C2C12, a mouse skeletal muscle cell line to investigate the effect of sera from septic rats on GR activity with an MMTV promoter containing a reporter plasmid in transient transfection assays. Figure 2 shows that sepsis induced GR-dependent transcription at all the time points tested from 2–16 h in C2C12 myocytes, indicating that induction of the transcriptional activity of the GR by sepsis occurred in both L6 and C2C12 myocytes.

Although the septic model used in the present study is clinically relevant and has been frequently employed to investigate the effect of sepsis on metabolic changes in skeletal muscle, it was important to determine if the increase in GR transcription was specific for CLP. We therefore measured GR transcription in L6 myocytes treated with sera from endotoxaemic rats. Sera from endotoxaemic rats were collected 16 h after the subcutaneous injection of 12.5 mg/kg of endotoxin. Similar to treatment with sera from septic rats, treatment with sera from endotoxaemic rats significantly increased GR transcriptional rate 5-fold (P < 0.01, sera from endotoxaemic rats compared with sera from vehicle control). The dose of endotoxin used was based on previous reports in which it induced sepsis-like metabolic changes in mice [9].

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Figure 3

(A) Plasma corticosterone levels were measured by RIA in sera from rats obtained at 2, 4, 6, 8 and 16 h following sham-operation (○) or CLP (●). (B) Plasma corticosterone levels in serum from rats obtained at 16 h following vehicle (open bar) or endotoxin (closed bar) administration. Results are means ± S.E.M. (n = 7 per group). The time point at 0 h represents normal rats. *P < 0.01 compared with the other groups by ANOVA.

GC-induced transcription of GR

The sepsis-induced GR gene expression is, at least in part, via a GC-mediated pathway [9], suggesting that GCs possibly regulate the GR at the transcriptional level. Therefore we performed experiments to elucidate the potential role of GCs in the sepsis-induced increase in GR gene transcription. Sepsis resulted in elevated plasma corticosterone levels and this effect of sepsis was seen as early as 2 h after CLP (Figure 3A). Although plasma corticosterone levels decreased in septic rats after 6 h, they still remained significantly higher than in sham-operated rats throughout the experimental period. The time course with regard to GR transcription (Figure 1A) was the same as that for plasma corticosterone levels. Plasma corticosterone levels were increased at 16 h in endotoxin-treated rats (Figure 3B), findings similar to previous reports [29a]. To assess the role of GCs in sepsis-induced changes in GR transcription, L6 myocytes were treated with various concentrations of DEX or CORT as indicated in Figure 4. Both of these GCs increased levels of GR transcription in a concentration-dependent manner, suggesting that GC up-regulation of GR transcription is not specific to a particular GC in skeletal muscle. These observations support our hypothesis that sepsis-induced GR transcription is GC-dependent.

Sepsis enhanced GC-induced GR transcription

Since sepsis increases not only a ligand for GR, but also other cytokines [30,31], cofactors [32,33] and transcriptional factors [34,35], we examined whether the sepsis-induced transcription of the GR is GC-dependent. We found that DEX treatment increased the activation of the MMTV promoter by 8-fold; furthermore, the enhancement of activation of the MMTV promoter was significantly higher by the treatment of a combination of DEX and sera from septic rats than DEX alone (results not shown), suggesting that other factors, including tumour necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6, which are increased in sera from septic and endotoxaemic animals [20,31,36], might be involved in sepsis-induced transcription of the GR gene in the skeletal muscle.

RU 38486 inhibited the GC-like activity of sepsis

To examine further the role of GCs in increased GR transcription, L6 myocytes were treated with RU 38486 in the presence of either GC or sera from septic rats. This treatment prevented the increase in GR transcription levels seen during treatment with sera from septic rats (Figure 1A) and abolished the DEX-induced MMTV transcription (Figure 5). Thus we conclude that sepsis-induced GR transcription is at least partly GC-dependent and inhibited by RU 38486.
Figure 5  Effect of RU 36486 on sera from septic rats on GR
L6 myocytes were transfected with pMMTV–Luc and pCMV–SPORT–β-gal plasmids. (A) L6 myocytes were cultured with ethanol (EtOH) or 1 µM DEX in the absence or presence of 1 µM RU 38486 (RU). (B) L6 myocytes were cultured with sera from sham-operated or septic rats in the absence or presence of 1 µM of RU 38486 (RU). Luciferase activities were calculated as in the legend to Figure 1. Results are means ± S.E.M. of three independent experiments. * P < 0.01 compared with the other groups by ANOVA.

Effect of sepsis on GR-dependent transcription is not due to promoter specificity
To test if the effect of sepsis on GR transcription is promoter-specific, we used another GR-dependent reporter plasmid [ptk-(GRE)2–Luc], which contains two palindromic GREs [37], in our transient transfection assays. Treatment with sera from septic rats significantly induced this promoter in L6 myocytes 6-fold, which is similar to the effect observed with pMMTV–Luc. In conclusion, our results show that sepsis acts to positively regulate GR transcription under a series of different circumstances.

Effect of GR on muscle proteolysis in septic rats
To test further the role of GCs in the regulation of muscle proteolysis, septic or sham-operated rats were treated with either DEX or RU 38486. In initial experiments, rats were treated with 10 mg/kg of DEX or RU 38486, doses that have been shown in previous studies [8] to stimulate or block muscle proteolysis respectively. Figure 6 shows that DEX enhanced the sepsis-induced increase in both total and myofibrillar protein breakdown, whereas RU 38486 inhibited these effects. These findings confirm further that GC-mediated sepsis-induced muscle proteolysis is via the GR.

DISCUSSION
Although numerous studies have focused on the effects exerted by GCs on the GR and its gene, examinations of the regulation of the transcription of the GR gene in skeletal muscle are rather sparse. In the present study, we have examined the effect of sepsis on GR transcription in skeletal muscle to test our hypothesis that increased levels of the GR protein in septic muscle may be the result of the increased production of the GR, which is regulated at the transcriptional level. We used sera from CLP-induced septic rats (a well-established model that has been widely used to study sepsis-related metabolic changes [8,9,16–19]) to treat L6 myocytes, thereby establishing...
a novel in vivo model for study of the molecular mechanism of GC-mediated muscle cachexia under septic conditions. Treatment with sera from septic rats resulted in increased GR gene transcription in two different cell culture lines of skeletal muscle in a GC-dependent manner, as demonstrated in myocytes treated with DEX or CORT and RU 38486. Our data are consistent with the concept that, during sepsis, the production and amount of GR is increased in skeletal muscle and that these effects of sepsis result in increased GR gene expression via regulating gene transcription. In the present study, treatment of rats with DEX enhanced, whereas RU 38486 blocked, the sepsis-induced increase in muscle proteolysis, which is consistent with in vivo experiments in the rat showing that sepsis-induced muscle proteolysis is mediated by GCs via GR gene expression [9].

Muscle proteolysis induced by sepsis, cancer, burns, diabetes, denervation and AIDS pose a challenge that appears to be closer to a solution with the discovery of GC-like activities during sepsis [38,39]. In the present study, we demonstrate that sera from septic rats induce the transcriptional activation of a GR-dependent promoter in skeletal muscle cell lines, which is consistent with our recent data [9] showing that sepsis up-regulates the GR in rat skeletal muscles. Although it has been argued that the GC-like activity of sepsis may vary with species, cell type or treatment condition [9], based on our findings, we consider it very unlikely that any one of these arguments can explain the discrepancy in the sepsis-induced GR transcription for the following reasons. First, we used myocytes derived from mouse and rat sources, including the same L6 myocytes that were used previously in studying effects of GCs on the GR [25], and showed that GR transcription was increased in both cell types by treatment with sera from septic rats, suggesting that it is not cell-type-specific. Secondly, we transfected two different GR-responsive promoter vectors, including MMTV and GRE, and both showed a response to sera from septic rats, which indicates there is no promoter specificity in this effect. Thirdly, we compared treatment with sera from septic and endotoxemic rats and found increased plasma GCs and muscle protein degradation in both conditions [38] and both treatments showed a response to GR-dependent promoter, thus excluding the possibility of condition specificity. Finally, we used a well-documented antagonist of the GR, RU 38486 [6], and showed that the effect of sera from septic rats on the GR was blocked in the presence of this antagonist, thus supporting the results we obtained with DEX treatment (Figure 4). Thus the discrepancy of sepsis on GR gene transcription could be explained by GC-dependence. Collectively, there is now firm evidence from this in vitro study and the previous in vivo report [9] that sepsis up-regulates GR expression via gene transcriptional regulation in skeletal muscle. Therefore GCs must act as mediators in the up-regulation of GR transcription under septic conditions, which is evidenced by a dramatic increase in plasma corticosterone in septic rats and that sepsis-induced up-regulation of GR transcription was also prevented by treatment with RU 38486 (Figure 5). Therefore up-regulation of the GR by in vivo sepsis or by sera from septic rats in vitro in skeletal muscle is GC-dependent.

Other data have shown previously that cAMP and prostaglandin E2 up-regulate GR expression in human articular chondrocytes [40], and IL-1β, TNF-α and IL-6 up-regulate GR-binding activity in human lymphoid, monocyteid and hepatoma cell lines [41]. The stimulation of GR expression by sepsis in rat skeletal muscle in vivo is well known [9]; however, to our knowledge, any direct effect of sepsis on GR transcription in skeletal muscle has not been explored in vitro. Our present study is the first report demonstrating that sepsis increases GR transcription mediated by GCs in skeletal muscle. We used sera from septic rats at 2–16 h following CLP, since GC levels were increased as early as 2 h (Figure 3A) and muscle protein breakdown rate was highest in septic rats at 16 h after CLP treatment [8–10]. Although we have evidence to ascertain the mechanisms by which sera from septic rats accomplishes its effect on GR transcription in skeletal muscle, via GCs, it does not exclude other factors that are increased by sepsis. Data available in the literature show that GCs either up- or down-regulate GR transcription [42]. Cytokines such as IL-1β, TNF-α and IL-6 have also been reported to increase GR-binding activity in human monocytic (U937), lymphoid (CESS) and hepatoma-derived (HepG2) tumour cell lines [41], IL-2 and IL-4 increase GR number in both T-and non-T-cells [43], IL-1β, TNF-α and IL-6 all increased in septic conditions [20,31,36]. However, the molecular mechanism of how these cytokines regulate the GR under septic conditions is not well understood. Our results show that sera from septic rats synergize DEX-up-regulated GR transcription in L6 myocytes. Consequently, any possible actions observed between these cytokines on GC levels might be due to changes in receptor expression of these cytokines, which remains to be elucidated. We hypothesize that sepsis-induced GR-binding activity may be regulated by GCs and cytokines. Although the difference between GC-induced down-regulation of GR-binding activity in L6 myotubes [25] and up-regulation in rat skeletal muscle in vivo [9] could be explained by the increase in cytokines after GCs administration in rats, further studies are needed to determine whether cytokines are involved in regulating GR-binding activity under septic conditions and what their role is, and whether sera from septic rats regulate GR-binding activity in the L6 myotubes.

Since sepsis induces nuclear factor κB (involved in the molecular pathways used by IL-1β, TNF-α and IL-6) [44,45], as well as other nuclear transcriptional factors, such as AP-1 [45] and CCAAT/enhancer-binding protein
[46], the role of these transactivation factors in GR gene regulation cannot be excluded either. According to findings reported previously [2,8,39], the induction of the muscle proteolysis pathway by sepsis, including the ubiquitin–proteasome, Ca2+-dependent and liposomal-mediated pathways, and the subsequent increase in circulating steroids, cytokines and transcriptional factors [30–35] can be explained by the activation of the GR. In addition, it is possible that sepsis-induced GR gene expression could be regulated by protein–protein interactions between GRE and nuclear factor κB or and AP-1 or and CCAAT/enhancer-binding protein at the level of gene transcription regulation. Although the mechanism by which GCs activate gene expression within the proteolysis pathways and lead to muscle protein breakdown under septic conditions is not known, it is well known that GCs can activate gene transcription by binding to cytosolic GR and forming a complex which translocates to the nucleus. In the nucleus, this complex acts as a transcriptional activator by binding to a GRE in the promoter of a target gene. It has been analysed that E214K, a proteasome enzyme, contains a GRE-binding site in its promoter [45]. Therefore the present observations are important, because they support the role of GCs in the regulation of the catabolic response in skeletal muscle during sepsis, offer a clue to linking GCs and the proteolysis pathways and provide further elucidation of the molecular mechanism by which the GR is regulated in muscle proteolysis in this new model of treatment of myocytes with sera from septic rats.

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