Expression and binding activity of the glucocorticoid receptor are upregulated in septic muscle

E. SUN, D. FISCHER, T. PRITTS, C. WRAY, AND P. HASSELGREN

Department of Surgery, University of Cincinnati, and Shriners Hospitals for Children, Cincinnati, Ohio 45267

Received 30 August 2001; accepted in final form 23 October 2001

Sepsis is associated with multiple metabolic changes in skeletal muscle, including a pronounced increase in protein breakdown (50), inhibition of amino acid uptake and protein synthesis (22), efflux of glutamine resulting in reduced glutamine levels (5), and stimulated glutamine synthetase activity (1). These changes contribute to the muscle cachexia that is typically seen during prolonged and severe sepsis, both in patients (51) and experimental animals (25).

Previous studies provided evidence that glucocorticoids mediate several of the metabolic consequences of sepsis. Thus plasma glucocorticoid levels were elevated during sepsis (21) and a number of the metabolic changes were blocked by treatment with a glucocorticoid receptor (GR) antagonist (21, 49) or by adrenalectomy (29).

In addition to an increase in glucocorticoid levels, another mechanism by which glucocorticoids may account for the regulation of the metabolic consequences of sepsis could be an increase in the GR hormone binding activity caused by either increased receptor ligand affinity or increased availability of hormone receptor binding sites (36). The influence of sepsis on the expression and hormone binding activity of the GR in skeletal muscle is not well understood. The present experiments were designed to examine the effect of sepsis in rats on the expression and hormone binding activity of the GR in skeletal muscle. In addition, we tested the role of glucocorticoids in sepsis-induced changes in muscle GR because other studies suggest that glucocorticoids may regulate their own receptor in different tissues and cell types (26, 32).

MATERIALS AND METHODS

Animals and experimental model. Sepsis was induced in male Sprague-Dawley rats (40–60 g) by cecal ligation and puncture (CLP) as described previously (21, 25, 49, 50). The cecum was punctured twice with an 18-gauge needle. Control rats underwent sham operation, i.e., laparotomy and manipulation but no ligation or puncture of the cecum. All rats were resuscitated with saline (10 ml/100 g body wt) administered subcutaneously on the back at the time of surgery to prevent hypovolemia and septic shock. Rats had free access to drinking water after the surgical procedures, but food was withheld to avoid any influence of differences in food intake between the groups of rats on the GR expression and binding activity. Unless stated otherwise, the extensor digitorum longus (EDL) and soleus muscles were harvested 16 h after sham operation or CLP and immediately frozen in liquid nitrogen. Both a white, fast-twitch (EDL) and a red, slow-twitch (soleus) muscle were studied here, because previous studies provided evidence that the metabolic response to sepsis may be different in different types of skeletal muscle (25). The muscles were stored at −70°C until analysis of the expression and hormone binding activity of the GR.

The septic model used here has been used in several previous reports from both our and other laboratories to study sepsis-related metabolic changes (7, 21, 25, 49, 50). The model is clinically relevant because it results in hyperdynamic, hypermetabolic sepsis 16–18 h after CLP and it resembles the situation in patients with sepsis caused by intra-abdominal abscess and devitalized tissue. We and ot-
ers reported previously that rats are in a hyperdynamic, hypermetabolic phase of sepsis 16 h after CLP, as evidenced by hemodynamic and hematological changes (7, 34). Young, growing rats (age 4 wk) were used here to make possible comparisons with previous reports in which we examined the influence of sepsis on protein metabolism in incubated muscles from rats of a similar age (21, 25, 49, 50). Muscles from rats of this size are small enough to allow for measurement of protein turnover rates during incubation in vitro (23). In other studies we found evidence that sepsis-induced metabolic changes in skeletal muscle were similar in small, growing rats and adult rats (54) and that protein breakdown was regulated by similar mechanisms in muscle from septic patients (51) as in muscles from septic rats of the same size as used here.

To assess the role of glucocorticoids in sepsis-induced changes in GR expression and hormone binding activity, sham operation or CLP was performed in rats treated with the GR antagonist RU-38486 (35). Rats were treated by gavage with 10 mg/kg of RU-38486 (Research Biochemicals International, Natick, MA) or a corresponding volume of vehicle 2 h before sham operation or CLP as described in detail previously (21, 49). In previous experiments, this treatment prevented several sepsis-induced metabolic changes in skeletal muscle, including increased protein breakdown (21) and upregulated expression and activity of the ubiquitin-proteasome proteolytic pathway (49). Rats were treated with only one dose of RU-38486, because the half-life of the drug is relatively long (10–20 h) (11). Results from previous experiments suggest that the biological effects of RU-38486 last for at least 16–18 h in rats of the same age as used here (21, 49).

In additional experiments, endotoxemia was induced in rats by the subcutaneous injection of 12.5 mg/kg of endotoxin (Escherichia coli endotoxin O111: B4; Calbiochem, La Jolla, CA). Control rats were injected with corresponding volume (0.5 ml) of sterile saline. Muscles were studied 16 h after injection of endotoxin or saline.

**Cultured L6 myotubes.** L6 rat skeletal muscle cells were purchased from the American Type Culture Collection (Rockville, MD). The cells were grown to differentiated myotubes in six-well culture plates as described in detail previously (53). The purpose of these experiments was to test the effect of withdrawal of dexamethasone on GR hormone binding activity. Myotubes were treated with 1 μM dexamethasone for 3 h, whereafter they were washed three times in medium without dexamethasone and cultured for 3 h in the absence of dexamethasone. Control myotubes were cultured for 6 h in the absence of dexamethasone. After the 6-h experimental period, myotubes were harvested for determination of GR hormone binding activity (see below). The concentration of dexamethasone used in these experiments was based on previous studies in which treatment of cultured L6 myotubes with 1 μM dexamethasone per 6 h resulted in increased protein degradation (53).

**GR binding activity.** To increase the amount of tissue, muscles from four rats were pooled for each hormone binding assay. Muscles were homogenized in 3 vol of 10 mM Tris buffer (pH 7.5) containing 0.25 M sucrose, 1.5 mM EDTA, 10 mM sodium molybdate, 10 mM monothioglycerol, 2 mM p-nitrophenylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. The homogenates were centrifuged at 100,000 g for 60 min, and the supernatants (cytosolic proteins) were used for hormone binding assay as described by Al-Mohaisen et al. (3). The cytosol was treated with dextran-coated charcoal for 15 min at 4°C to remove any excess of endogenous steroid. The supernatant was then centrifuged at 300 g for 10 min. Aliquots of the supernatants were incubated in dupli- cate with different concentrations of [3H]dexamethasone (0.02–50 nM) for 24 h at 4°C with or without a 100-fold excess of unlabeled dexamethasone for nonspecific and total binding, respectively. [3H]dexamethasone binds specifically to the GR and has been used in several previous studies to determine GR binding activity (13, 14, 39, 44). Incubations were terminated by the addition of dextran-coated charcoal (0.5% charcoal and 0.05% dextran) to separate bound and free hormone. The mixture was centrifuged, and radioactivity was measured in the supernatant. Specific binding was determined by subtracting nonspecific binding from total binding. In initial experiments in which incubations were performed up to 42 h, specific binding reached steady-state levels after incubation for 20 h (data not shown). Data were subjected to Scatchard analysis (40) to differentiate between changes in receptor affinity and changes in amounts of hormone binding sites. Protein was determined according to Lowry et al. (28).

**GR protein levels.** GR protein levels were determined by Western blotting of muscle cytosolic extracts prepared as described above. For the hormone binding assay, using a rabbit polyclonal anti-human GR antibody as primary antibody and peroxidase-conjugated goat anti-rabbit IgG as a secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). β-Actin levels were determined by Western blotting to control for equal loading of the lanes by using a rabbit monoclonal anti-β-actin antibody as primary antibody and peroxidase-conjugated goat anti-rabbit IgG as a secondary antibody (Sigma, St. Louis, MO). Aliquots of the muscle extract (100 μg protein) were loaded on a 4–20% Tris-glycine gel (Novex, San Diego, CA) and transblotted onto nitrocellulose-enhanced chemiluminescence membranes (Amersham International, Buckinghamshire, UK). Membranes were blocked overnight with 10% nonfat dry milk in Tris-buffered saline, pH 7.6, containing 0.05% Tween 20 (TTBS) for 1 h and then incubated with the primary antibody at room temperature for 45 min. After being washed twice in TTBS, the blots were incubated with the secondary antibody for 15 min. The blots were then washed in TTBS for 5 min × 3, in Tris-buffered saline, pH 7.6, for 5 min, incubated in enhanced chemiluminescence reagents (Amersham Life Sciences), exposed on radiographic film (Eastman Kodak, Rochester, NY), and quantitated by densitometry. The levels were expressed as arbitrary units based on GR/β-actin ratios.

**GR mRNA levels.** GR mRNA levels were determined by Northern blot analysis. A cDNA template for the coding region of the rat GR (1,035 bp) (30) was synthesized by RT-PCR. The sequences of the PCR primers were as follows: GR sense 5′-CAT TAC GGG GTG CTG ACA TGT G-3′; GR antisense 5′-TCA TTT TTG ATG AAA CAG AAG-3′. After purification, cDNA fragments were subcloned into pGEM3. cRNA probes were synthesized with in vitro transcription reaction by using T7 RNA polymerase and [32P]-UTP (DuPont, DuPont) and stored at −80°C until used. RNA was quantitated by spectrophotometry and 20 μg was fractionated by electrophoresis on a 0.9% denaturing agarose-formaldehyde gel. The RNA was transferred to nylon membranes (Micro Separation, Westboro, MA) according to the manufacturer’s instructions. A cDNA probe for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was subject to random labeling with [32P]-dCTP (DuPont) as described previously (50, 51).

Total RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform method (8) using an RNA Stat-60 kit (Tel-Test “B,” Friendwood, TX) and stored at −80°C until used. RNA was quantitated by spectrophotometry and 20 μg was fractionated by electrophoresis on a 0.9% denaturing agarose-formaldehyde gel. The RNA was transferred to nylon membranes (Micro Separation, Westboro, MA) that were baked at 80°C under vacuum for 2 h. After prehybridization for 2 h at 55°C in buffer containing 50% formamide, 0.1% pyrophosphate, 50 mM Tris-Cl (pH 7.5), 5 × SSC (1 ×...
RESULTS

Binding of $[^3H]$dexamethasone in representative EDL and soleus cytosolic extracts prepared from sham-operated and septic rats is shown in Figs. 1 and 2. GR specific binding activity was determined by subtracting nonspecific binding from total binding and the maximum specific receptor binding was calculated for each muscle extract. Nonspecific binding was variable and ranged from 21 to 62% of total binding.

Basal GR specific binding activity in sham-operated control rats was higher in the EDL than in the soleus muscle. A similar difference between EDL and soleus rat muscles was reported by others as well (44). Sepsis resulted in increased specific hormone binding activity in both muscles 16 h after CLP with the relative increase being greater in soleus muscle (Fig. 3). Because GR binding activity was expressed as femtomoles per milligram cytosolic protein, it may be argued that the apparent increase in binding activity reflected reduced protein content in muscle from septic rats, rather than a true increase in hormone binding activity. This is not likely, however, because in a previous study we found that total protein content was not changed in soleus muscle and was decreased by ~20% in EDL 16 h after CLP in rats (20). Because sepsis-induced muscle cachexia mainly reflects degradation of myofibrillar proteins (24, 25), cytosolic protein was probably reduced to a much smaller extent. Thus the changes in GR binding activity noted here (~70% increase in EDL and ~2-fold increase in soleus muscle) were substantially larger than could be explained by changes in muscle protein content. To make certain that the results shown in Fig. 3 were not significantly influenced by reduced protein content in septic muscles, we also calculated GR binding activity per wet weight in the same experiment. Expressed in this unit, GR binding activity was 5.34 ± 0.34 and 7.47 ± 0.62 fmol/g wet wt in EDL muscles from sham-operated and septic rats, respectively ($P < 0.05$). The corresponding results in soleus muscles were 2.91 ± 0.18 and 5.48 ± 0.66 fmol/g wet wt ($P < 0.05$). Thus the relative increase in GR binding activity in septic muscle was similar when the calculation of binding activity was based on muscle cytosolic protein content or wet weight.

Increased specific hormone binding activity may be caused by increased ligand-receptor affinity, increased availability of binding sites, or a combination of these changes. Scatchard analysis showed that the ligand-receptor affinity (represented by the slope of the curve in the Scatchard plot) was not altered in septic muscle, but that the number of available binding sites (represented by the x-axis intercept) was higher in septic than in control muscles (Fig. 4). The same mechanism accounted for the increased hormone binding activity in both EDL and soleus muscle. The dissociation constant values (an index of ligand-receptor affinity) ranged from 1.11 to 1.17 nM for EDL muscles and from 1.10 to 1.20 nM for soleus muscles, with no significant differences between muscles from sham-operated and septic rats.

Increased availability of receptor binding sites can be the result of conformational changes of existing hormone receptors or an increased amount of the receptor. To examine the effect of sepsis on GR levels, we...
next determined the amount of muscle GR protein 16 h after sham operation or CLP by Western blotting. GR protein levels were increased during sepsis in both EDL and soleus muscle (Fig. 5). Similar to the specific binding activity, basal GR protein levels were higher in EDL than in soleus muscle, and the relative increase seen during sepsis was greatest in soleus muscle. An increased amount of the GR may be caused by upregulated synthesis or decreased degradation of the receptor or a combination of these changes. To assess the potential contribution of stimulated synthesis to the increased amount of GR, mRNA levels for the GR were determined by Northern blot analysis. Sepsis resulted in upregulated GR mRNA levels in both EDL and soleus muscle, and this effect of sepsis was seen 8 h after CLP and persisted throughout the remainder of the experimental period (Fig. 6). These results suggest, but do not prove, that the increased amount of GR protein levels in septic muscle may be the result of increased production of GR, possibly regulated at the transcriptional level.

We next performed experiments to elucidate the potential role of glucocorticoids in the sepsis-induced increase in GR expression and hormone binding activity. Sepsis resulted in elevated plasma corticosterone levels, and this effect of sepsis was seen already 4 h after CLP (Fig. 7). It should be noted that plasma corticosterone levels decreased in septic rats after the 4-h time point (although they remained significantly higher than in sham-operated rats throughout the experimental period). Thus the time course with regard to GR protein and mRNA levels (see Fig. 6) was different than the time course for plasma corticosterone levels. This observation could be interpreted in different ways. First, it is possible that the sepsis-induced changes in GR expression and activity were not caused by the elevated plasma corticosterone levels. Second, the fact that GR protein and mRNA levels were not increased until 8 h after CLP may reflect a time lag required for the increased corticosterone (noted already at 4 h) to result in increased GR expression. Finally, it may be speculated that high ACTH levels were involved in the upregulation of muscle GR expression. For example, if the plasma corticosterone levels decreased from 4 to 8 h despite persistent high levels of ACTH (possibly secondary to reduced sensitivity of adrenals to ACTH stimulation), the increased GR expression may have been caused by persistently elevated ACTH levels (although there is no report in the literature suggesting that ACTH regulates muscle GR expression). To elucidate the potential role of ACTH in the present findings, we measured plasma ACTH levels at different time points after sham operation or CLP. Results from that experiment showed that the time courses for plasma corticosterone and ACTH levels were similar, with no evidence of persistent elevation of ACTH levels after the 4-h time period (Fig. 7).
To further examine the role of glucocorticoids in the increased expression and activity of the GR, rats were treated with the GR antagonist RU-38486. This treatment prevented the increase in GR protein and mRNA levels seen during sepsis (Fig. 8) and abolished the sepsis-induced increase in GR binding activity (Fig. 9).

Although the results shown in Figs. 7–9 support a role of increased glucocorticoid levels in the upregulation of muscle GR expression and activity, an additional explanation may be that the increase in GR expression and activity was caused by the relatively sharp decline in plasma corticosterone levels seen between 4 and 8 h after CLP (see Fig. 7). To test the influence of a sudden withdrawal of glucocorticoids on GR binding activity, we subjected cultured L6 myotubes to treatment with 1 μM dexamethasone for 3 h, whereafter the cells were rinsed and cultured for an additional 3-h period in the absence of dexamethasone. The withdrawal of dexamethasone did not result in increased GR binding activity; the GR binding activity was 209 ± 18 fmol/mg protein in control myotubes and 225 ± 21 fmol/mg protein in myotubes treated with dexamethasone for 3 h followed by culture in dexamethasone-free medium for 3 h (n = 6 in each group; not significant), suggesting that the increase in muscle GR expression and activity seen in rats 8 and 16 h after CLP did not reflect the decline in plasma corticosterone levels noted after the 4 h time point.

Although the septic model used in the present experiments 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 hormone binding activity was specific for CLP. We therefore next measured GR binding activity as well as protein and gene expression in EDL muscles of endotoxemic rats. Endotoxemia induced by the subcutaneous injection of 12.5 mg/kg of endotoxin resulted in increased GR hormone binding, protein, and mRNA levels (Fig. 10). The dose of endotoxin used here was based on previous reports in which it induced sepsis-like metabolic changes in mice (52).

Fig. 4. Scatchard analysis of GR-dexamethasone binding data in EDL (A) and soleus (B) muscles. Muscles were studied 16 h after sham operation (○) or CLP (■). Each data point is the mean from 3 experiments with pooled muscles from 4 rats in each experiment.

Fig. 5. GR protein levels determined by Western blot analysis in EDL (A) and soleus (B) muscles 16 h after sham operation or CLP. Representative Western blots are shown in each panel. Western blots were quantitated by densitometry, and results are means ± SE with n = 6 or 7 in each group. *P < 0.05 vs. sham by Student’s t-test.
DISCUSSION

In the present study, we examined the effect of sepsis on the expression and hormone binding activity of the GR in skeletal muscle. Sepsis, induced by CLP in rats, resulted in increased GR binding activity in different types of skeletal muscle, and Scatchard analysis provided evidence that the increased hormone binding activity reflected increased availability of binding sites. Because Western and Northern blot analyses showed that both protein and mRNA levels for the GR were increased in septic muscle, our data are consistent with the concept that during sepsis, the production and amount of GR are increased in skeletal muscle and that these effects of sepsis result in increased GR hormone binding activity.

The present observations are important because they support the role of glucocorticoids in the regulation of the catabolic response in skeletal muscle during sepsis (1, 23, 49). In previous studies, we found that sepsis- and burn-induced muscle proteolysis could be blocked by treating rats with the GR antagonist RU-38486 (16, 21). In additional experiments, treatment of septic rats with RU-38486 blocked the upregulation of ubiquitin gene expression and energy-dependent protein breakdown (49), providing further evidence for the important role of glucocorticoids in sepsis-induced muscle cachexia. The results in the present study suggest that glucocorticoids are essential for the catabolic response in skeletal muscle not only because the hormone levels are increased but also because the amount and hormone binding activity of the GR are increased.
in skeletal muscle during sepsis. Although the data reported here do not provide a definitive link between unregulated GR expression and metabolic changes in septic muscle, taken together with results reported previously (16, 21, 49), the data are consistent with the concept that sepsis-induced muscle cachexia is regulated by both increased plasma levels of glucocorticoids and upregulated expression and activity of the GR.

The results in the present study were unexpected for several reasons. First, increased glucocorticoid levels, as reported during sepsis in previous studies (19, 21) and in the current experiments as well, are usually associated with downregulated expression and ligand binding activity of the GR (6, 9, 12, 33, 38, 41, 43, 48). It should be noted, however, that receptor expression and activity are not universally downregulated in situations characterized by increased hormone levels. For example, when mice were subjected to different types of stress, the number of GRs was not reduced (47), and the authors of that study concluded that, although glucocorticoids can downregulate GR number, the physiological significance of this process is uncertain as receptor number does not appear to be downregulated by endogenous adrenal glucocorticoids in the intact animal. When rats were subjected to immobilization stress, hepatic cytosolic GR levels were increased together with a substantial increase in plasma corticosterone levels (3). A similar upregulation of the GR was noticed in denervated skeletal muscle (37). This is important because denervated muscle is characterized by increased protein breakdown, similar to sepsis. In other reports, chronic endogenous hypercortisolism in humans did not result in downregulation of the GR (27, 42), further supporting the concept that there is not always an inverse relationship between hormone levels and receptor activity. In the present study, increased corticosterone levels in septic rats not only failed to reduce, but even increased, GR expression and binding activity. A positive regulation of the GR by glucocorticoids was reported by other authors in hepatocytes (4), human T cells (15), and in cultured human myeloma cells (17). Thus the regulation of the GR by glucocorticoids may vary in different cell types and tissues under different experimental conditions.

A second reason why the present results were unexpected is the fact that in previous studies, GR amount and binding activity were reported to be decreased in skeletal muscle during sepsis and endotoxemia. For example, when Stith and McCallum (45) induced endotoxemia in mice, GR binding activity was reduced by 50% in skeletal muscle (although it is not known from that study in which muscle GR hormone binding was measured). In another study, Ali et al. (2) induced sepsis in rats by the subcutaneous injection of live *E. coli* bacteria and found that the GR hormone binding activity declined by ~40% in hindlimb skeletal muscle.
The protein levels and gene expression of the GR in skeletal muscle were not reported in those studies. The reason(s) for the apparent discrepancy between the present results and those reported previously (2, 45) are not known but may be related to differences in animal age and species and/or septic models. The experimental model used in the present study, CLP in rats, is clinically relevant because it resembles the situation in patients with septic peritonitis caused by devitalized tissue and intra-abdominal abscess and the model reproducibly results in increased muscle protein breakdown and upregulated expression and activity of the ubiquitin-proteasome proteolytic pathway (21, 25, 49, 50), similar to the catabolic response in patients with sepsis (51). It should be noted that although the present results differed from previous studies in septic and endotoxemic muscle, increased GR binding activity has been reported in other conditions characterized by increased glucocorticoid levels and muscle atrophy, including limb immobilization (13, 31) and muscle denervation (14, 37). Interestingly, in those studies as well, increased GR hormone binding activity reflected an increase in the number of receptors with unchanged affinity.

An additional unexpected finding in the present study was that GR expression and binding activity were increased in both soleus and EDL muscle. In previous studies, we found that the increase in protein breakdown during sepsis was particularly pronounced in the white fast-twitch EDL muscle, with much less pronounced sepsis-induced changes in the red slow-twitch soleus muscle (25). The present observations suggest that the upregulated GR expression and hormone binding activity in septic muscle are important not only for stimulated proteolysis but for other metabolic consequences of sepsis as well. Interestingly, glucocorticoid-dependent changes in glutamine metabolism during sepsis are not limited to fast-twitch muscle but also occur in red slow-twitch muscle (1, 5).

The mechanism of the upregulated GR expression and binding activity noticed in septic muscle in the present study is not fully understood, but results from the experiments in which sepsis was induced in rats treated with RU-38486 strongly suggest that the elevated glucocorticoid levels played a significant role. The important role of glucocorticoids in the catabolic response to sepsis and other conditions as well, including burn injury, cancer, and starvation (16, 23), is supported by increased glucocorticoid levels in these conditions and by the prevention of muscle cachexia by adrenalectomy or treatment with the GR antagonist RU-38486 (16, 21, 49). The present results are important because they suggest that during sepsis, not only are glucocorticoid levels increased but muscle may also be more sensitive to the hormone because of increased numbers of the GR.

In addition to increased protein breakdown, catabolic muscle is characterized by a number of other metabolic alterations, and there is evidence that at least some of these are regulated by glucocorticoids. For example, the production of glutamine is substan-

Fig. 10. GR binding activity (A), protein (B), and mRNA levels (C) in EDL muscles 16 h after the subcutaneous injection of 12.5 mg/kg of endotoxin or corresponding volume of sterile saline. Results are means ± SE with n = 7 in each group. *P < 0.05 vs. saline by Student’s t-test.

The protein levels and gene expression of the GR in skeletal muscle were not reported in those studies. The reason(s) for the apparent discrepancy between the present results and those reported previously (2, 45) are not known but may be related to differences in animal age and species and/or septic models. The experimental model used in the present study, CLP in rats, is clinically relevant because it resembles the situation in patients with septic peritonitis caused by devitalized tissue and intra-abdominal abscess and the model reproducibly results in increased muscle protein breakdown and upregulated expression and activity of the ubiquitin-proteasome proteolytic pathway (21, 25, 49, 50), similar to the catabolic response in patients with sepsis (51). It should be noted that although the present results differed from previous studies in septic and endotoxemic muscle, increased GR binding activity has been reported in other conditions characterized by increased glucocorticoid levels and muscle atrophy, including limb immobilization (13, 31) and muscle denervation (14, 37). Interestingly, in those studies as well, increased GR hormone binding activity reflected an increase in the number of receptors with unchanged affinity.

An additional unexpected finding in the present study was that GR expression and binding activity were increased in both soleus and EDL muscle. In previous studies, we found that the increase in protein breakdown during sepsis was particularly pronounced in the white fast-twitch EDL muscle, with much less pronounced sepsis-induced changes in the red slow-twitch soleus muscle (25). The present observations suggest that the upregulated GR expression and hormone binding activity in septic muscle are important not only for stimulated proteolysis but for other metabolic consequences of sepsis as well. Interestingly, glucocorticoid-dependent changes in glutamine metabolism during sepsis are not limited to fast-twitch muscle but also occur in red slow-twitch muscle (1, 5).

The mechanism of the upregulated GR expression and binding activity noticed in septic muscle in the present study is not fully understood, but results from the experiments in which sepsis was induced in rats treated with RU-38486 strongly suggest that the elevated glucocorticoid levels played a significant role. The important role of glucocorticoids in the catabolic response to sepsis and other conditions as well, including burn injury, cancer, and starvation (16, 23), is supported by increased glucocorticoid levels in these conditions and by the prevention of muscle cachexia by adrenalectomy or treatment with the GR antagonist RU-38486 (16, 21, 49). The present results are important because they suggest that during sepsis, not only are glucocorticoid levels increased but muscle may also be more sensitive to the hormone because of increased numbers of the GR.

In addition to increased protein breakdown, catabolic muscle is characterized by a number of other metabolic alterations, and there is evidence that at least some of those are regulated by glucocorticoids. For example, the production of glutamine is substan-

The protein levels and gene expression of the GR in skeletal muscle were not reported in those studies. The reason(s) for the apparent discrepancy between the present results and those reported previously (2, 45) are not known but may be related to differences in animal age and species and/or septic models. The experimental model used in the present study, CLP in rats, is clinically relevant because it resembles the situation in patients with septic peritonitis caused by devitalized tissue and intra-abdominal abscess and the model reproducibly results in increased muscle protein breakdown and upregulated expression and activity of the ubiquitin-proteasome proteolytic pathway (21, 25, 49, 50), similar to the catabolic response in patients with sepsis (51). It should be noted that although the present results differed from previous studies in septic and endotoxemic muscle, increased GR binding activity has been reported in other conditions characterized by increased glucocorticoid levels and muscle atrophy, including limb immobilization (13, 31) and muscle denervation (14, 37). Interestingly, in those studies as well, increased GR hormone binding activity reflected an increase in the number of receptors with unchanged affinity.

An additional unexpected finding in the present study was that GR expression and binding activity were increased in both soleus and EDL muscle. In previous studies, we found that the increase in protein breakdown during sepsis was particularly pronounced in the white fast-twitch EDL muscle, with much less pronounced sepsis-induced changes in the red slow-twitch soleus muscle (25). The present observations suggest that the upregulated GR expression and hormone binding activity in septic muscle are important not only for stimulated proteolysis but for other metabolic consequences of sepsis as well. Interestingly, glucocorticoid-dependent changes in glutamine metabolism during sepsis are not limited to fast-twitch muscle but also occur in red slow-twitch muscle (1, 5).

The mechanism of the upregulated GR expression and binding activity noticed in septic muscle in the present study is not fully understood, but results from the experiments in which sepsis was induced in rats treated with RU-38486 strongly suggest that the elevated glucocorticoid levels played a significant role. The important role of glucocorticoids in the catabolic response to sepsis and other conditions as well, including burn injury, cancer, and starvation (16, 23), is supported by increased glucocorticoid levels in these conditions and by the prevention of muscle cachexia by adrenalectomy or treatment with the GR antagonist RU-38486 (16, 21, 49). The present results are important because they suggest that during sepsis, not only are glucocorticoid levels increased but muscle may also be more sensitive to the hormone because of increased numbers of the GR.

In addition to increased protein breakdown, catabolic muscle is characterized by a number of other metabolic alterations, and there is evidence that at least some of those are regulated by glucocorticoids. For example, the production of glutamine is substan-
tially increased in catabolic muscle, at least in part reflecting glucocorticoid-regulated increases in the expression and activity of glutamine synthetase (1). In contrast, reduced muscle uptake of amino acids during sepsis does not seem to be regulated by glucocorticoids (55), suggesting that this hormone does not account for all metabolic changes seen in catabolic muscle.

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-37908 and by Grant 8580 from the Shriners of North America, Tampa, FL. D. R. Fischer was supported by a Research Fellowship from the Shriners of North America and T. A. Pritts by National Institutes of Health Training Grant 1732-GM-008478.

REFERENCES


33. Okret S, Poel linger L, Dong Y, and Gustafsson JA. Down-regulation of glucocorticoid receptor mRNA by glucocorticoids and recognition by the receptor of a specific binding sequence within a receptor cDNA clone. Proc Natl Acad Sci USA 83: 5899–5903, 1986.


39. Santoro JC, Harris G, and Sitlani A. Colorimetric detection of glutamine synthetase-catalyzed transerase activity in glu-


